The effect of Bt-transformation and various environmental factors on the volatile emission of maize: Potential influence on direct and indirect defense against herbivores

Dissertation

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1. General Introduction

"For plants, sometimes a good offense is the best defense." May Berenbaum

1.1. Defense strategies of plants

We live in a world that is dominated by plants, although they can be attacked by microorganisms, fungi, or herbivorous insects, all of which can cause severe damage. Hence, plants developed elaborate and effective defense strategies against their enemies. In addition to very obvious mechanisms like thorns, spikes and hairs that protect the plant from attack by mostly large herbivores and also can affect the ability of insects to walk on or bite into a plant (Roessingh and Städler, 1990; Bernays and Chapman, 1994), plants produce an enormous and diverse range of substances which are called secondary metabolites. These substances do not participate directly in growth and development and were a long time thought to be waste products and "present only accidentally" (Kossel, 1891). Contrary to primary metabolites, which can be found in all plants and enable essential metabolic processes, secondary metabolites differ in their distribution among plant kingdom and often are present in specific taxa only.
The structural diversity of these phytochemicals such as terpenoids, alkaloids or phenylpropanoids is the most remarkable characteristic of plant secondary metabolism. Each of these major groups consists of several thousand different compounds identified to date (Croteau et al. 2000). Since chemicals can be effective at low concentrations and small modifications in structure may dramatically enhance biological activity, this strategy of using chemicals as defensive compounds may be less energy consuming than other mechanisms such as morphological structures. Moreover, the rapid response potential, the variety of regulatory mechanisms and the diversity of steps in biosynthetic pathways enable plants to deploy chemical defense options rapidly (Berenbaum and Zangerl, 1999). Phytochemicals can act in different ways to insects: toxins can poison feeding insects; substances mimicking insect hormones can negatively affect their development and growth; deterrents can prevent feeding or oviposition by insects; and inhibitors of digestion can decrease the nutrient value of food by reducing nutrient uptake.
Nevertheless, chemical defense also causes physiological costs to plants by diverting resources and energy from growth, development and reproduction. Hence, it would be advantageous for the plants to induce chemical defence when required (Baldwin and Preston, 1999). To optimise physiological costs and the basic necessity of being guarded against herbivores and pathogens, plants are able to induce the production of defensive chemicals after an attack. This induced response might either act as a bitrophic system that directly reduces herbivory impact by producing toxic compounds (bottom-up control), or can indirectly influence the fitness of an attacker by attracting a third trophic level such as predators or parasitoids (top-down control). One example is the group of acacia that provide shelter (leaf domatia) or food in the form of extrafloral nectar to ants, thus recruiting permanent bodyguards, which prevent insects from feeding by keeping them off the plant (Heil et al., 2001). A more frequent indirect defense mechanism might be the emission of volatiles that attract natural enemies of attacking herbivores and lead them to the site of damage. While predators directly kill their prey and consequently stop further food consumption by the herbivore, the benefit for the plant by luring parasitoids which lay their eggs into the herbivore without killing it instantly is less apparent. However, Hoballah & Turlings (2001) showed that parasitized caterpillars keep on feeding, but often at decreased levels. Moreover, there might be a long-term effect, which is beneficial for the plant populations.

1.2. Volatile organic compounds as a signal of induced indirect defense

The release of volatiles from by herbivore-attacked plants is found in a number of ecosystems but has mainly been reported for crop plants. It was first demonstrated for maize seedlings where the damage by leaf-consuming caterpillars elicits the emission of volatiles, which are not emitted when the plant is unharmed and serve as attractants for the parasitoid *Cotesia marginiventris* (Turlings et al., 1990; see figure 1.1). In lima bean, Dicke et al. (1990) identified the volatiles emitted after infestation by sucking spider mites, which were attractive for predatory mites. Also, for apples the emission of volatiles attractive to predatory mites after damage by sucking spider mites has been demonstrated (Llusia & Penuelas, 2001). An infestation of cotton plants by folivorous caterpillars induced the plants to emit volatile compounds, which attracted hymenopteran parasitoids of the lepidopteran larvae (McCall et al., 1994). This type of indirect defense could also be shown for parasitoids and caterpillars on cabbage (Mattiacci et al., 1994) and cotton (Röse et al., 1997). Not only herbivore feeding, but also oviposition can induce the release of attracting volatiles, as shown by Meiners and Hilker (2000) for the elm – elm leaf beetle – beetle egg parasitoid system. Recently, Rasmann et al. (2005) reported on a below-ground tritrophic system. Here, maize roots that were damaged by larvae of *Diabrotica virgifera*, released (*E*)-β-caryophyllene thus recruiting entomopathogenic nematodes.

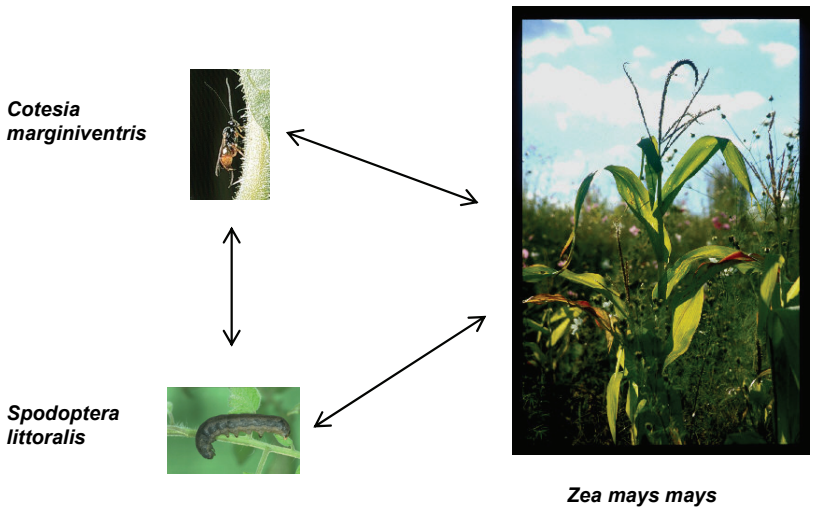

Figure 1.1. Tritrophic system, consisting of the corn plant *Zea mays*, the pest insect *Spodoptera littoralis* and the parasitic wasp *Cotesia marginiventris*

Although the quantity and composition of the herbivore-induced volatile blends of plants show considerable variations, they usually include four groups of chemicals: 1) green leaf volatiles including saturated and unsaturated six-carbon alcohols, aldehydes, and esters, which are derived from the lipoxygenase pathway and are generally released after mechanical wounding (Turlings et al., 1998a); 2) terpenoid volatiles like mono- and sesquiterpene olefins and alcohols as well as homoterpenes, which are generated via isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP) in the mevalonic acid pathway (MVA) or the methylerythritol phosphate pathway (MEP); 3) indole, a secondary amine derived from tryptophan (Donath and Boland, 1994), and 4) methyl salicylate and other benzoic acid derivatives, which are synthesized via the shikimate/ phenylalanine pathway.

1.2.1. Variation and specificity of induced volatiles

Many different factors have an effect on the quantitative and qualitative composition of the volatile mixture. Not only plant species, but also cultivars of a single species can vary in their volatile blend as shown for eleven maize cultivars and their wild ancestor teosinte (Gouinguene et al., 2001) as well as for wild cotton and several cotton cultivars (Loughrin et al. 1995). Also, abiotic factors can influence the volatiles emitted from a plant. It has been reported for corn plants that nutrient availability as well as climatic conditions such as soil humidity, air humidity, or temperature also can affect the release of volatile organic compounds (Gouinguene and Turlings, 2002). Furthermore, developmental stages and the organs of a plant determine the composition of a volatile blend as Köllner et al. (2004) showed for corn. Also, different feeding habits of herbivores can influence the release of volatiles. Takabayashi et al. (1995) found considerable qualitative differences between maize plants which were attacked by young and old *Pseudaletia separata* caterpillars. On the other hand, a similar study with corn and different larval stages of *Spodoptera littoralis* showed neither quantitative nor qualitative differences in the volatile blend (Gouinguene, 2003).
Volatile compounds should act as very specific and reliable signals for host- or prey-seeking insects, giving the parasitoid or carnivore information about herbivore species, developmental stage, and number of herbivores. Several studies have shown that the application of herbivore oral secretion to mechanical wounds induced the same volatile pattern as herbivore feeding (e.g. Halitschke et al., 2001, Mattiacci et al., 1994). Obviously these secretions contain substances, which induce the herbivore-specific volatile release. Several of such elicitors have been identified from herbivore regurgitant such as a β-glucosidase from *Pieris brassicae* (Mattiacci et al., 1995), fatty acid-amino acid conjugates from *Manduca sexta* (Halitschke et al., 2001), or volicitin [N-(17-hydroxylinolenyl)-L-glutamine] from *Spodoptera exigua* (Alborn et al., 1997).

1.2.2. Induced volatile emission in maize

The tritrophic interaction consisting of maize, folivorous caterpillars and hymenopteran parasitoids is one of the most intensively studied indirect defense systems since it was first described by Turlings et al. (1990). After damage by lepidopteran larvae, the plant emits a complex volatile blend, which is different to that released after mechanical wounding and serves as a cue for the generalist parasitoid *Cotesia marginiventris.* The parasitoid thereby orients towards plant-derived volatiles to locate its host (Turlings et al., 1989). After laying its eggs into the caterpillars, the parasitoid develops inside the host and thus leads to reduced feeding activities of the caterpillar.
Upon wounding, maize plants emit green leaf volatiles such as (*Z*)-3-hexanal, (*E*)-2-hexanal, (*Z*)-hexen-1-ol, and (*Z*)-3-hexen-1-yl acetate whose amounts drop after 0.5 to 2h. Two hours after wounding, the first induced terpenes are released like the monoterpene linalool and the homoterpene (3*E*)-4,8-dimethyl-1,3,7,-nonatriene (DMNT). Most of the sesquiterpenoid compounds including the herbivory-induced (*E*)-β-caryophyllene, (*E*)-α-bergamotene, and (*E*)-β-farnesene as well as indole appear in the volatile blend 4 to 6h after damage (Turlings et al., 1998a). Beside the duration of feeding by the herbivore, the time of the day also seems to play a prominent role in the emission of the volatile blend: it is mainly released during day light hours with a peak around noon when the parasitoids are most active and declines at dusk or when herbivory ceases. It has been shown that not only the total amount of volatiles but also the composition of the blend is important for attracting the parasitoid (Hoballah et al., 2002). Within the volatile profile, the terpenoid

fraction may have an important function as the terpenoid compounds are the domint components of the blend and are closely correlated with herbivore damage. Consequently, these compounds might serve as reliable cues for host-seeking parasitoids because of their complex pattern and high variability, which is a premise for a highly specific signal.

1.2.3. Terpenes, the largest group of secondary metabolites

The terpenes constitute the largest class of secondary products with more than 30000 compounds identified and described (Buckingham, 1998; Croteau, 2000). In general, terpenes are insoluble in water. Regardless of their high number and structural variability, all terpenes are formed by the fusion of five-carbon elements with a branched carbon skeleton of isopentenyl diphosphate (IPP) and dimethyl-allyl diphosphate (DMAPP). Since all terpenes share a basic building block, a C_5-unit with an isoprenoid structure, this class of secondary metabolites belongs to the large group of isoprenoids referring to their biogenetic origin. Terpenoids are classified by the number of C_5-units they are composed of. Ten-carbon terpenes, initially thought to be the smallest naturally occurring terpenoids, contain of two C_5-units and are called monoterpenes. 15-carbon terpenes are called sesquiterpenes (three C_5-units), and 20-carbon terpenes with four C_5-units are called diterpenes. Triterpenes consist of six (C_{30}), tetraterpenes of eight (C_{40}) and polyterpenes of more than eight isoprene-units.
In plants, the C_5-units can be synthesized from primary metabolites via two different pathways: the cytosolic mevalonic acid pathway (MVA) and the methylerythritol phosphate pathway (MEP) found in the plastids (Lichtenthaler et al., 1997; Rohmer et al., 1993). Later on isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) join to form geranyl diphosphate (GPP), the 10-carbon precursor for monoterpenes. An additional molecule of IPP can be linked to GPP to form the 15-carbon unit farnesyl diphosphate (FPP), which is the precursor of the sesquiterpenes. The precursor of the diterpenes, geranylgeranyl diphosphate (GGPP) can be formed by the reaction of IPP with FPP. Finally, FPP and GGPP can dimerize to form precursors for triterpenes and tetraterpenes, respectively.
Despite the broad range of functions of isoprenoids in primary metabolism, such as hormones (gibberellins, absisic acid, cytokinins), photosynthetic pigments (carotenoids, phytol), electron carriers (ubiquinone), or structural components of membranes (phytosterols), isoprenoid compounds of the secondary metabolism such as terpenes are mainly involved in defense against herbivores, pathogens, or in competing plants. Terpenes might act in various ways on insects such as through the inhibition of ATP formation, disruption of molting hormone activity, or interference with nervous systems (Langenheim, 1994; Gershenzon and Kreis, 1999). All these different mode of actions may result in strong toxic or feeding deterrent effects on feeding insects. In conifers like pine and fir, inducible and constitutive terpene-based defense mechanisms evolved by which insect and fungal invaders are fended off due to the accumulation of terpenes in resin ducts in the trunk, needles, and twigs (Franceschi et al., 2005). Many plants such as peppermint, lemon, basil or sage contain mixtures of volatile terpenes, called essential oils, leading to the characteristic odor of their foliage. These essential oils or single constituents have insect repellent properties (e.g. Gonzalez-Coloma et al., 1995) but also are important in flavoring foods and producing perfumes. Other functions of terpenoid compounds are their activity as signals in the indirect defense of plants (e.g. de Moraes et al., 1998; Dicke et al., 1990; Heil, 2004; Rasmann et al., 2005) and their mediating role in the interaction of plants with their pollinators and seed dispersers (Dudareva and Pichersky, 2000; Pichersky and Gershenzon, 2002).

1.3. Scope of the thesis

Although terpenoid compounds are a well-investigated group of secondary metabolites, known to fulfill numerous biological functions, many open questions about their roles within ecosystems remain to be elucidated. This thesis is aimed at increasing knowledge about the function of these compounds in the direct and indirect defense of corn plants under controlled conditions as well as in a field situation and whether the transformation of corn with a Bt-coding gene might interfere with the plants' ability to cope with abiotic and biotic stress factors.

In this thesis, the following topics will be adressed:

1.3.1. Effects of Bt-endotoxin on the volatile release of maize plants in the laboratory and in the field

This study, part of a large-scale project on the safety research of Bt-maize in agriculture, was set to elucidate whether the transformation of maize lines with a Bt-coding gene could change their volatile profile and thus disturb the tritrophic system consisting of corn, lepidopteran larvae and parasitic wasps. The Bt-toxin is known to rapidly kill the larvae of the target insect *Ostrinia nubilalis* (European cornborer/ ECB) and as a consequence, the specific volatile blend induced by these larvae might not be emitted. On the other hand, non-target insects such as the Egyptian cotton leaf worm *Spodoptera littoralis* or the turnip moth *Agrotis segetum* are less susceptible to the Bt-toxin and may, due to their different feeding habit or the presence of special elicitors in their regurgitant induce another volatile pattern in the infested maize plants. To clarify this question, the volatile blend of corn plants transformed with the Bt-transformation events MON810 or Bt176 was collected in the laboratory and compared to that emitted by the corresponding isogenic control plants. In additional experiments the same cultivars were screened in two different fields over three growing seasons and the volatile blend was compared to that found in the laboratory. Also, differences between plant stages, years and field sites were investigated. To evaluate the effect of the volatile blend on host-seeking parasitoids, a method established by Thaler (1999) was used to examine parasitation rates of caterpillars in the field.

1.3.2. The role of maize terpenes in direct defense against insect herbivores

Terpenes are widespread plant secondary metabolites that are frequently implicated in interactions of plants with insects. Many studies have shown that herbivory by lepidopteran larvae can induce the formation of complex mixtures of volatile terpenes which serve to attract parasitic wasps that use the larvae as hosts. In addition, for many terpenoids it was shown that they are directly active against insects for example β-selinene, which was proved to be toxic to mosquito larvae (Momin et al., 2000). However, it is still unclear if the terpene mixtures in maize can also serve as direct defenses against herbivores, as toxins or feeding deterrents. To examine this, 12 terpenoids, which are produced either constitutively in mature corn plants or after herbivore infestation, were mixed in an artificial diet and tested in different concentrations on the generalist herbivore *Spodoptera littoralis*. Different parameters indicating insect fitness such as maximum weight, mortality, developmental day of pupation, duration of pupation and emergence success were compared among control and experimental treatments.

1.3.3. The impact of abiotic and chemical stress on the volatile emission of corn plants

In the field experiments, the climatic conditions and also the terpene blend of the corn plants differed considerably among the three years. A laboratory study by Gouiguene and Turlings (2002) showed that abiotic factors might change the volatile emission of corn seedlings. Recently, Vuorinen et al. (2004) reported that ozone-exposure of lima bean induced the emission of volatile compounds. To explain the field data, the influence of several abiotic factors such as drought, high temperature, constant flooding and ozone treatment were tested on the volatile emission of corn plants in the laboratory. Additionally, the herbicide bromoxinyl, which is known to induce oxidative stress was applied to the corn plants and the effect on the volatile blend was estimated. According to Köllner et al. (2004) development and organ effects influence the composition of a volatile blend emitted by corn plants. Bromoxinyl was applied to three different developmental stages to test wether the qualitative change in the volatile profile found in the field can be attributed to different life stages.

This thesis is arranged in chapters according to the different research questions outlined above. Chapter 2 deals with the effect of Bt-endotoxin on the volatile release of maize plants in the laboratory and in the field. Studies on the direct defense of corn plants and the impact of corn-produced terpenes on a generalist herbivore are described in chapter 3. Different abiotic factors and their influence on the emission of volatile organic compounds are illustrated in chapter 4. Finally, in chapter 5 and chapter 6 the results of chapters 2-4 are discussed and summarized.

2. Effects of Bt-endotoxin on the volatile release of maize plants in the laboratory and in the field

Safety research and monitoring methods for the agricultural production of Bt-maize, Joint project sponsored by the Federal Ministry of Science and Education (BMBF) and BEO-Jülich

2.1. Introduction

The most commonly cultivated transgenic plants with endogenous Bt-toxin are cotton and corn. Transgenic cotton (*Gossypium hirsutum*) is cultivated on 9 billion hectare, mainly in the USA, China, and India, but also in Columbia, Mexico, Argentina, Australia, South Africa, and Indonesia. Maize with endogenous Bt-toxin is cropped in the USA were it represents up to 46 % of the total corn cultivation. Furthermore, it is cultivated in Canada, Argentina, South Africa, Honduras, Bulgaria, the Philippines, and since 2004 also in Spain. In some EU-countries release experiments with a parallel safety monitoring have been conducted in France, Spain, Italy, Belgium, Germany, Hungary, and the Czech Republic. Moreover, the Bt-toxin has been introduced to other plants such as sugarcane, tomato, walnut, citrus fruits, sunflowers, coconuts, lentil, cabbage, apples, or persimmon, but the transformants are not commercially cultivated (public available informations on http://www.transgen.de).

The Bt-toxin is a natural occurring protein, which is synthesized by the soil bacterium *Bacillus thuringiensis* and used since the 1940's in organic cultivation by spray application to protect crop plants from insect herbivores. Nonetheless, a lot of laboratory studies on the effect of Bt on non-target and target organisms were performed (e.g. Hilbeck et al., 1999; Zwahlen et al., 2000; Hilbeck, 2001; Jesse and Obricky, 2004; Meissle et al., 2005; Vojtech et al., 2005) since a study found toxic effects of Bt-toxin on the caterpillars of the popular monarch butterflies (Losey et al., 1999). In feeding tests with the lepidopteran larvae *Spodoptera exigua* a decreased fitness was found with a lower pupal weight and longer developmental time when they were exposed to a Bt-containing diet (Stapel et al., 1997).

Possible risks of plants with the endogenous Bt-toxin on an ecosystem have to be elucidated and minimized before transgenic plants can be cultivated regularily in the field. There are several possibilities by which the Bt-toxin can influence an ecosystem: 1) the soil fauna might be affected through an accumulation of the Bt-toxin in the soil (e.g. Point et al., 2005). Hopkins et al. (2005) demonstrated that a part of the Bt-toxin ingested by primary decomposers is not digested and is released in the active form into the soils, thus probably influencing the soil fauna. 2) non-target insects can be killed directly or at least disturbed in their development by feeding on Bt-containing tissues or collecting transgenic pollen 3) the Bt-toxin can affect predators or parasitoids of herbivores which feed on the transgenic plant either by being directly toxic to the predator or parasitoid or by indirect effects since the fitness of its host or prey is reduced (Schuler et al., 1999; Vojtech et al., 2005; Meissle et al., 2005). A study by Atwood et al. (1997) showed an indirect effect on the emergence of the parasitoid *Cotesia marginiventris*, which were reared on *Heliothis virescens* larvae exposed to several concentrations of Bt-toxin. Contrary, Ludy and Lang (2006) reported that the garden spider *Araneus diadematus* (Clerck) was not affected by pollen from transgenic maize plants. These spiders eat their webs in order to "recycle" the material and thus might come into contact with transgenic pollen when the webs are placed near corn fields. However, the application of the

insecticide Baythroid, which is the only insecticide that is allowed to be used against *O. nubilalis*, negatively affected the fitness of the garden spiders.

2.1.1. The Bt-toxin

The Bt-toxin present in transformed corn plants can be found in the soil bacterium *Bacillus thuringiensis* (Huebner). During sporulation this gram-positive bacterium produces crystal proteins of ~130 kDa that are dissolved in the insect midgut after ingestion because of the alkaline environment to protoxins. These protoxins can further be truncated by gut proteases to the 65-70 kDa form. This activated toxin binds to specific receptors on the membranes of the gut epithelial cells, inserts itself into the membrane and kills the cells by colloid osmotic lysis during spore formation. As these receptors only can be found in insects, no other group of animals can be killed directly by the Bt-toxin.
The gene responsible for the formation of the Bt-toxin can be introduced into a plant with the help of the bacterium *Agrobacterium tumefaciens* via the Ti-plasmid, which can be transferred to plant cells under certain conditions. Another way of transforming a plant is to enhance the permeability of the cell membrane. This can be done by chemicals, by electroporation, or with a DNA-bomb. To ensure that the endogenous Bt-toxin is only produced in special tissues, it is necessary to control the gene by a tissue-specific promoter thus leading to the synthesis of the toxin in roots, leaves or stalk and not in the pollen. Approximately 170 natural occurring Bt-toxins are known which act on different groups of insects. In corn plants, different types of Bt-toxins were introduced such as Cry 1Ab, Cry 1Ac or Cry 9c that differ both in the length of the gene and the promoter used (publ. available information on http://www.transgen.de and http://www.biosicherheit.de).

2.1.2. Target- and non-target lepidopterans

The indirect defense of a plant should be very specific to give the prey- or host-seeking insect reliable information on insect species or number of herbivores. As reported by De Moraes et al. (1998) for tobacco, maize, and cotton plants, distinct volatile mixtures were produced after feeding of the two closely related herbivores *Heliothis virescens* and *Helicoverpa zea*. Otherwise, corn plants emitted qualitatively the same volatile blend after attack by the folivorous caterpillar *Spodoptera littoralis* and the stemborer *Ostrinia nubilalis*, but quantitative differences exist (Turlings et al., 1998b). In contrast, even heavy infestation with the aphid *Rhodalosiphum maidis* did not induce a measurable emission of volatiles (Turlings et al., 1998b). However most of the studies were conducted in the laboratory under controlled conditions on young plants. Thus, the indirect defense of maize seedlings induced by folivorous insects may be different in the field depending on the herbivorous species feeding on the plant. While the target-lepidopteran larvae *O. nubilalis* is killed by the endogenous Bt-toxin, non-target herbivorous insects such as *A. segetum* might still feed on the maize plant leading to a distinct volatile composition.

2.1.2.1. The European corn borer *Ostrinia nubilalis* (Huebner)

The European corn borer *O. nubilalis* (ECB) belongs to the family Noctuidae and is thought to have originated in Europe, where it is widespread. Furthermore it is distributed in Canada and the United States, and also occurs in northern Africa. The European corn borer usually goes through two generations per year although there may be a third generation in some years. The larvae of this insect attack field corn, sweet corn, and

popcorn as well as many other plants such as snap and lima beans, pepper, potato, and grasses, with a stem large enough for the larvae to enter. They feed on all above ground tissues of a plant and bore into, feed and tunnel within the tassel, ear, ear shank (figure 2.1.a), and stalk (figure 2.1.b and e) forming cavities that reduce the strength of the stalk and ear shank thereby predisposing the plants to stalk breakage and ear drop, and also interfere with the translocation of water and nutrients. Furthermore, the boring and tunneling by the European corn borer allows several fungi to enter the plant.

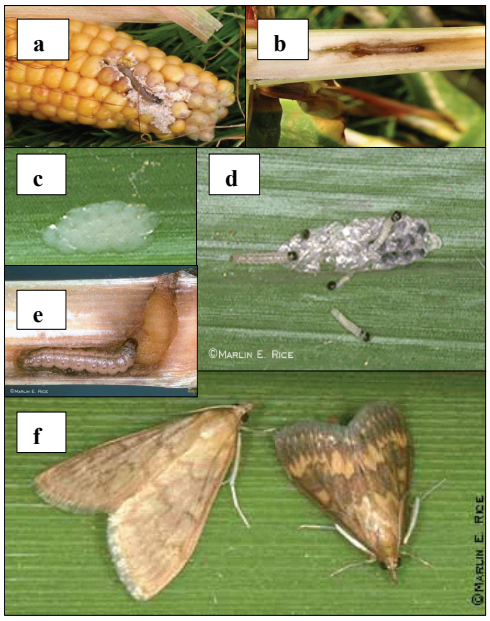

Figure 2.1. Life stages of the European corn borer *Ostrinia nubilalis* (Huebner)
a: Tunneling larva in the corn cob; b: tunneling larva in the stalk; c: freshly laid eggs; d: hatching larvae; e: tunneling larva; f: female (left) and male (right) moth
(Information and pictures were public available on http://entomology.unl.edu/ecb/ecb1.htm and http://creatures.ifas.ufl.edu/field/e_corn_borer.htm)

There are four life stages: egg, larva (borer), pupa and imago (moth). The larval stage goes through five instars and the full-grown fifth instar overwinters in corn stalks, cobs, and plant debris. The overwintered larvae change into pupae in spring and emerge as moths ten to fourteen days later during May and June. Within approximately two weeks, female moths lay egg masses of 15 to 25 eggs per day near the midrib on the underside of the leaves. Freshly laid eggs are white (see figure 2.1.c) and darken before hatching. The larvae hatch within five to seven days depending on temperature. Newly hatched larvae disperse (figure 2.1.d) and feed on the developing leaves in the whorl for the first two instars. Third-instar larvae leave the whorl, bore into stalks and excavate tunnels (cavities) where they complete their development (figure 2.1.e). Fifth-instar larvae of the first generation pupate in the cavities from which the second generation moths emerge in July and August (figure 2.1.f). Summer moths lay most of their eggs on the undersides of the ear leaf and three leaves above and below. After hatching, the larvae feed on the leaves and in leaf axils for some days, and then move behind the leaf sheaths or into ear tips. Third-instar larvae bore into the stalk, ear shank or ear. Usually these larvae do not pupate, and overwinter as larvae but in years of extended growing seasons a small percentage pupates and produces moths. Eggs laid by these moths are not of economic importance since the maize plants at this late date are beyond the period of susceptibility.
Among native predators that affect eggs and young larvae of the ECB in the United States are the insidious flower bug *Orius insidious* (Say) (Hemiptera: Anthocoridae), green

lacewings *Chrysoperla* ssp. (Neuroptera: Chrysopidae), and several ladybird beetles (Coleoptera: Coccinellidae). Although native predators often eliminate up to 20% of corn borer eggs or larvae, imported parasitoids seem to be more important. To the potentially important species belong *Lydella thompsonii* (Herting) (Diptera: Tachinidae) which may kill up to 30 % of second generation borers. Other parasitoids affecting corn borer populations are *Eriborus terebrans* (Gravenhorst) (Hymenoptera: Ichneumonidae), *Simpiesis viridula* (Hymenoptera: Eulophidae), or *Macrocentris grandii* (Goidanich) (Hymenoptera: Braconidae).

2.1.2.2. The Turnip moth *Agrotis segetum* (Denis & Schiffermüller)

The turnip moth belongs to the family Noctuidae and is distributed in Europe, Asia and South Africa. The larvae of these moths are polyphagous and feed on roots and leaves of crop plants such as cereals, potato, carrots, tobacco, vine, or maize, but also on wild plants such as couch-grass (*Agropyrum*), bindweed (*Convolvulus*), or plantain (*Plantago*).
Especially in Southern Europe this species is considered as a pest insect since it may heavily infest crop plants and vegetables by feeding on the roots (figure 2.2.a).

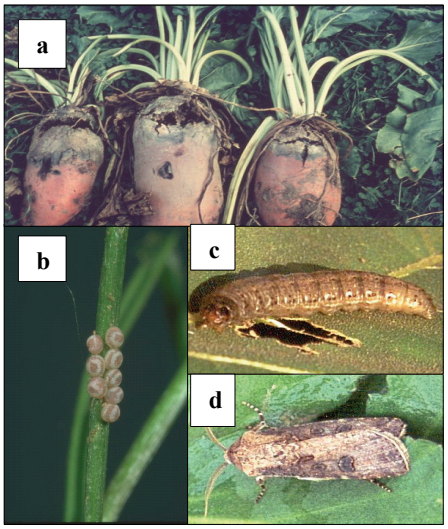

Figure 2.2. Life stages of the Turnip moth *Agrotis segetum* (Denis & Schiffermüller)
a: damage on beet; b: eggs on a stem; c: larva; d: adult moth
(Information and pictures were public available on http://de.wikipedia.org/wiki/Agrotis_segetum and http://www.inra.fr/Internet/Produits/HYPPZ/RAVAGEUR/6agrseg.htm)

Usually there are two generations per year with the larvae hibernating in different instars depending on the region and pupating in April. Fully-grown caterpillars are up to 50 mm long, have a greyish body with a reddish head and two parallel longitudinal lines in the middle region of the body (figure 2.2.c). Moths of the first generation fly from May to June and females deposit their up to 1200 eggs singly or in small clusters on the underside of wild plants, on stems (figure 2.2.b) as well as on the ground surface. After approximately 15 days, the larvae hatch and first feed on wild plants. Later on they attack the neighbouring cultivated species by damaging the foliage (figure 2.2.c) and cutting the petioles, mainly during night. Adults have dark-brown forewings with a spot in the middle (figure 2.2.d) and white and grey hindwings found in males and females, respectively. The moths of the second-generation can be seen from August to November. Larvae of this generation develop from August to the end of October and overwinter at different instars, but some produce a partial third generation in October-November.

2.1.3. Scope of this chapter

Although a lot of studies were conducted, which focus on possible risks of the release of genetically modified plants (GM plants) on non-target organisms, little information is available about the performance of GM plants in the field. The present study was carried out to clarify the question whether the transformation with a Bt-coding gene might have an influence on the tritrophic interaction between maize, non-target lepidopterans, and their parasitoids. Firstly, the volatile blend of transgenic maize plants could be altered due to the introduced gene or differential resource allocation. Secondly, as the target lepidopteran insect *Ostrinia nubilalis* is killed by the endogenous Bt-toxin, non-target insect species such as *Agrotis segetum* could damage the corn plants in the field and might change the volatile profile of the plants qualitatively or quantitatively due to different feeding habits or the presence of a special elicitor. Thus, the attractiveness of damaged plants for parasitic wasps might be influenced. To answer these open questions, several Bt-transformation events and their corresponding isogenic lines were left untreated or infested with the target lepidopteran *Ostrinia nubilalis* and the non-target lepidopterans *Agrotis segetum* and *Spodoptera littoralis*, and the volatile blend was collected and monitored for qualitative or quantitative differences. These laboratory experiments provide a good indication of the potential effects of transgenic plants but they do not give the complete information with respect to the complex environment found in the field. Hence, a mobile volatile collection system was developed, which allowed the collection of volatile organic compounds of the same cultivars in two different fields over three vegetation periods from 2001 to 2003. The volatile blends collected in the field were compared to those found in the laboratory and differences between plant stages and years were verified. To evaluate the effect of the volatile blend on host-seeking parasitoids, a method developed by Thaler (1999) was used to examine parasitation rates of caterpillars in the field.

2.2. Material and Methods

2.2.1. Plants

The maize cultivars used in this study were the transgenic line Novelis (MON810; Monsanto, Germany) and the near isogenic line Nobilis which did not express Bt-toxin. Furthermore, the pairs Prelude (isogenic)/ Valmont (Bt176; Syngenta Agro, Germany) and Antares (isogenic)/ Navares (Bt176; Syngenta) were examined (table 2.1). In field experiments near Halle (Saxony-Anhalt), the following varieties of *Zea mays* plants were used: Nobilis/ Novelis and Prelude/ Valmont. In the field near Kitzingen (Bavaria) Antares/ Navares as well as Nobilis/ Novelis were examined (table 2.1). The above mentioned lines were also cultivated and studied in the laboratory. Here, the plants were grown in individual plastic pots of 16 cm diameter in clay substrate potting soil (Lasmann, Gross-Hesepe, Germany) with Osmotic fertilizer (Scotts, Nordhorn, Germany) in a climate chamber (York Int., York, USA) set at 22 °C day/ 18 °C night, 65 % relative humidity, 1 mmol $(m^2)^{-1}s^{-1}$ of photosynthetic active radiation, and under a 16 h photoperiod. The terpene blend was collected from four-week old plants that were either uninduced or induced with *Spodoptera littoralis* (Boisd.), *Ostrinia nubilalis* (Huebner) or *Agrotis segetum* (Denis & Schiffermüller), respectively (table 2.1). For herbivory treatments, 4-5 third instar larvae were placed on a four-week old plant and enclosed by a cage made of petri dishes, in which circles were cut out and covered with gauze (Röse et al., 1996).

Table 2.1. Corn cultivars examined in this study

isogenic line	transgenic line	Bt-event	Field site	insects tested in the laboratory
Nobilis	Novelis	MON810	Halle, Kitzingen	*S. littoralis*
Prelude	Valmont	Bt176	Halle	*S. littoralis, A. segetum, O. nubilalis*
Antares	Navares	Bt176	Kitzingen	*S. littoralis, O. nubilalis*

2.2.1.1. Transformation events of the transgenic maize cultivars

The assayed transgenic maize lines express a *Bacillus thuringiensis* (Bt) toxin of the Cry1Ab toxin family. In MON810 (line Novelis), the toxin is 816 amino acid long (92kDa) and is mainly produced in green parts of the plants as well as in the roots, anthers, and the corn cob in the low µg-range per gram fresh weight. No toxin can be found in the pollen. In contrast, plants containing Bt176 transformation events (Valmont, Navares) possess a Bt toxin of 648 amino acid length (72.6kDa), which can be found in the lower µg-range in leaves and in ng-amounts in anthers, pollen and corn cob, respectively. In this line, no toxin is produced in the roots.

2.2.2. Insects

Eggs of the Egyptian cotton leaf worm, *S. littoralis* (Boisd.) were obtained from Syngenta (Basel, Switzerland) and reared on an artificial wheat germ diet (Heliothis mix, Stonefly Industries, Bryan, USA) for 10 to 15 days at 22°C under radiation of 750 µmol/$(m^2)^{-1}s^{-1}$. Eggs of the European cornborer (ECB) *Ostrinia nubilalis* (Huebner) and the turnip moth *Agrotis segetum* (Denis & Schiffermüller) were kindly provided by Prof. G.A. Langenbruch from the BBA Darmstadt and were reared on maize leaves under the same conditions as *S. littoralis* but in total darkness.

2.2.3. Field sites

2.2.3.1. Field site near Halle

The maize field near Halle had a size of approximately 22 ha and was surrounded by grassland as well as fields of sugar beet and wintergrain. An overview over the herbicides applied in the field is given in table 2.2. The field was divided into parcels of land with 6 replicates for each maize cultivar (figure 2.3). The parcels were arranged in random order to ensure a statistical reliability of the data. The soil was classified as loess and black/black brown soil rich in humus with a pH ~ 6.5. Contents of phosphorus, potassium and nitrate were in the optimal range. The isogenic line Nobilis in the parcels 3.x was treated with the cornborer-specific insecticide Baythroid once per vegetation period (figure 2.3), while the other cultivars remained untreated.
Temperature and relative humidity were recorded directly in the field before the volatile collections started and after all measurements were finished (Testo 625; Testo GmbH, Lenzkirch, Germany). Metereological data such as relative humdidity, rainfall, day temperature and ozone concentrations over the course of all field seasons were obtained from the DWD (Deutscher Wetterdienst; Potsdam, Germany). Climatic conditions near the field site as well as directly in the field are shown in tables A 1 – A 15 in the appendix.

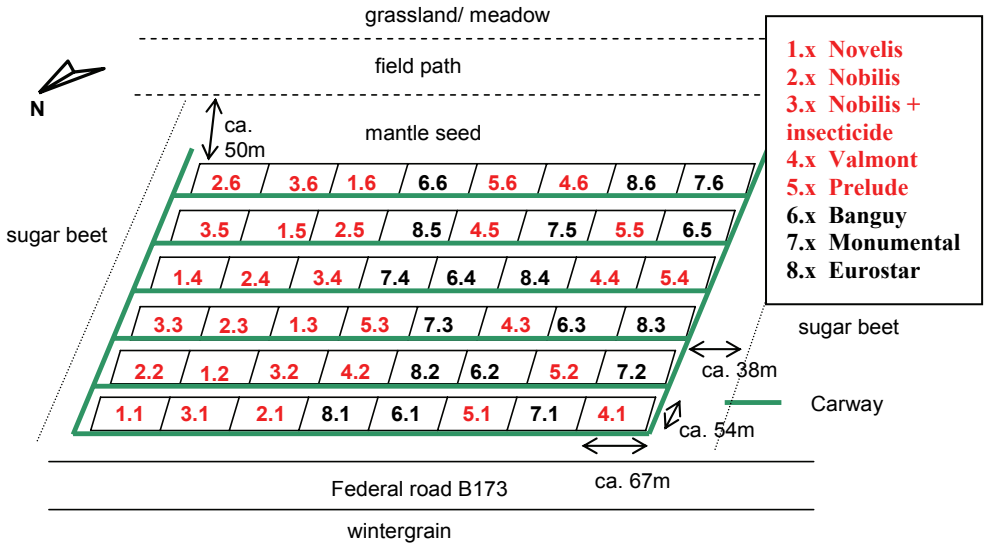

Figure 2.3. The field site near Halle (Saxony-Anhalt).
Examined cultivars are marked in red.

Table 2.2. Herbicides used in the field site near Halle

year	date of exposure	trade name	herbicides	site of action
2001	9.06.01	Zintan goldPack	S-metolachlor, mesotrione, tertbuthylazin	root and shoot inhibitor
2002	14.05.02	Cato	rimsulfuron	acetolactate synthase inhibitor
		Lido	pyridate and tertbuthylazin	photosynthase inhibitor (PS II, binding site C)
	31.05.02	Motivell	nicosulfuron	acetolactate synthase inhibitor
	3.06.02	Cato (varieties 4-8)	rimsulfuron	acetolactate synthase inhibitor
2003	21.05.03	Zintan goldPack	S-metolachlor, mesotrione, tertbuthylazin	root and shoot inhibitor
		Callisto	mesotrione	photosynthase inhibitor (PS II, binding site unknown)
		Cato (mantle seed and car ways)	rimsulfuron	acetolactate synthase inhibitor
		MaisTer	isoxadiphen-ethyl, iodo-sulfuron, foramsulfurone	herbicide safener, acetolactate synthase inhibitor
	10.06.03	Cato	rimsulfuron	acetolactate synthase inhibitor

2.2.3.2. Field site near Kitzingen

The field near Kitzingen was surrounded by crop fields and divided into eight parcels of which one half was treated with insecticide while the other was not (figure 2.4). To enhance the number of replicates, all parcels were subdivided into four subparcels of the same size. There is no information available on soil character, pH of soil or pesticides used in the field. An overview over climatic conditions near and directly in the field site is given in the tables A 16 – A 25 in the appendix.

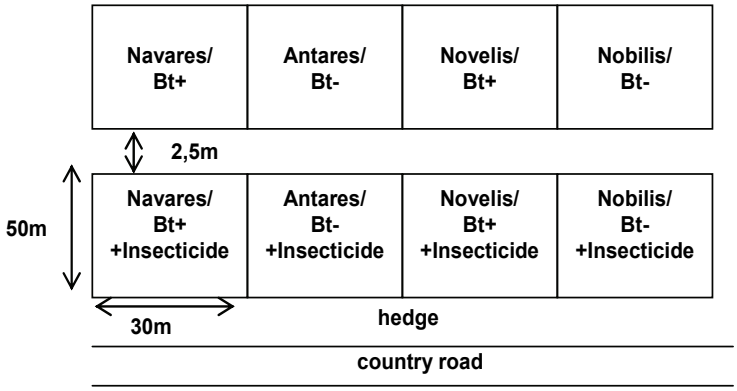

Figure 2.4. The field site near Kitzingen (Bavaria).

2.2.4. Measurements of terpene emission

2.2.4.1. Field equipment

To collect maize volatile blends in the field, a mobile volatile collection system was developed (see figure 2.5). Six independent volatile collection units were built to allow six parallel measurements.

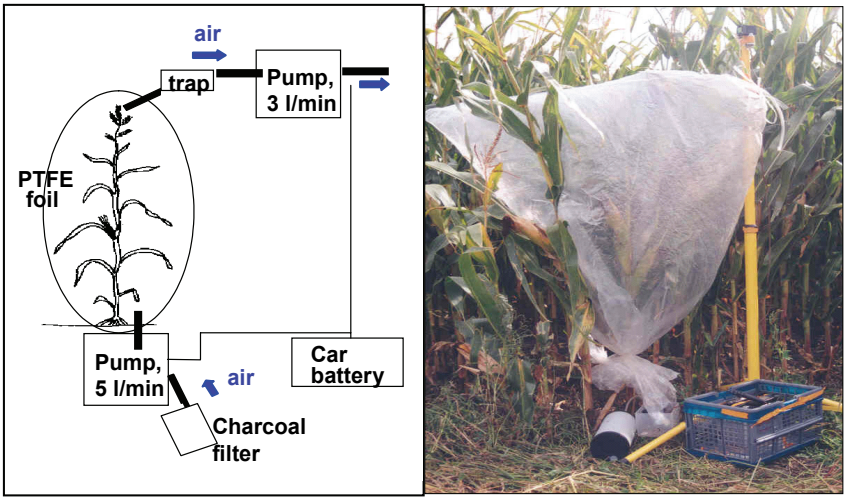

Figure 2.5. Field volatile collection unit

A corn plant was placed in a bag made out of 50 µm thin PTFE foil (custom-made product; Koch GeoPlan, Germany) which was then closed with a string tightened around the stem. External air cleaned by an activated charcoal filter (AUT-SCA-E2; Böcker Filtertechnik GmbH, Germany) was pumped into the bag at a rate of 5 l/min (pump type N86KNDC; KNF Neuberger, Freiburg, Germany). The air inside the plastic bag was then drawn with a second pump (type NMP50KNDC, KNF Neuberger) at a rate of 3 l/min via a volatile collection trap [(150 mm x 5 mm glass tubes containing 75 mg SuperQ (80/100 mesh; Alltech, Deerfield, Ill., USA)], which adsorbs many oxygenated and hydrocarbon compounds. The air in the bag surrounding the plant was at a slightly higher pressure than the environment, thus preventing contamination by outside volatiles. For further analysis, the traps were washed with 200 µl organic solvent (CH_2Cl_2) containing nonyl acetate as

internal standard (40 ng/µl). The eluted compounds were then separated by gas chromatography and identified by subsequent mass spectrometry.

2.2.4.2. Plant volatile collection in the field

At the field site near Halle, the collection of terpene emission was started when the plants had developed the third leaf (beginning to middle of June) and was repeated throughout the whole season, until the plants were harvested (end of September/ begin of October). In this time period, each parcel was examined once a month, three to four times throughout the whole season. In 2001, only the cultivars Novelis and Nobilis (parcels 1-3) were examined, while in the following years additionally the lines Valmont and Prelude (parcels 4 and 5) were monitored (see figure 2.3).
In Kitzingen, the released terpenes were collected from 13.-15.08.2002, 10.-12.07.2003 and 25.-27.08.2003.
All volatile collections were conducted between 10 am and 2 pm on days that were sunny or overcast. At the end of each measurement, temperature and relative humidity inside the plastic bag were recorded (Testo 625; Testo GmbH, Lenzkirch, Germany).

2.2.4.3. Plant volatile collection in the laboratory

To analyse the plant volatiles emitted under controlled conditions, an automated collection system was used that was described by Heath & Manukian (1994). The entire collection system was situated in a climate chamber (VB1014/S; Vötsch, Bahlingen-Frommern, Germany) where all environmental parameters can be controlled. The conditions were set to 22 °C, 16 h photoperiod with 750 µmol m^{-2} s^{-1} of photosynthetical active radiation, 75 % relative humidity during dark and 100 % relative humidity during light. The whole plant was placed in the chamber and the volatiles were trapped with SuperQ traps (Alltech), eluted with CH_2Cl_2 containing an internal standard (nonylacetate, 40 ng/µl) and further analysed by GC-MS.

2.2.4.4. GC-analysis (GC-MS, GC-FID)

Separation of compounds was performed on a Hewlett-Packard (Palo Alto, USA) 6890 gas chromatograph with He as carrier gas at a rate of 1 ml min^{-1}, a splitless injection mode (temperature 220 °C) and a injection volume of 2 µl. The standard temperature profile was programmed in the following way: initial temperature of 40 °C, holding for 3 min, than increasing by 5 °C min^{-1} up to 170 °C and increasing by 30 °C min^{-1} up to 240 °C (3-min hold). The GC was connected to a Hewlett-Packard 5973 quadrupole-type mass selective detector, which had an ionization potential of 70 eV, a scan range of 50-400 amu, a quadrupole temperature of 150 °C, and a transfer line and a source temperature of 230 °C. To quantify terpene amounts, GC-FID was applied. Compounds were first separated on a Hewlett-Packard 6890 gas chromatograph (carrier gas: He at 2 ml min^{-1}) under the same conditions described above and analysed on a flame ionisation detector (FID) at 250 °C. Hewlett-Packard Chemstation software was used to collect and process the data. Based on the comparison of peak areas with those of the internal standard nonyl acetate, the detected volatiles were quantified and differing relative response factors for standard, homo-, mono- and sesquiterpenes were determined according to the formulas in Scanlon and Willis (1985). Terpene compounds were identified by comparing their retention times and mass spectra with those of authentic standards (table 2.3).

Table 2.3. Standards for GC analysis and their origin

Compound	Standard	Origin
(E)-α-bergamotene	opoponax oil	Dracoco (Minden, Germany)
(E,E)-α-farnesene	volatile collection sample	C. Schnee (MPI CE, Jena, Germany)
α-selinene	celery oil	Roth (Karlsruhe, Germany)
(E)-β-caryophyllene	(E)-β-caryophyllene	Fluka (Buchs, Switzerland)
(E)-β-farnesene	farnesene	Fluka
β-bisabolene	bisabolene	Bedoukian (Danburry, USA)
(E)-β-ocimene	ocimene	Fluka
β-selinene	celery oil	Roth
β-sesquiphellandrene	Zingiber officinale	T.Köllner (MPI CE)
cycloisosativene	cycloisosativene	Fluka
δ-cadinene	δ-cadinene	Fluka
DMNT	volatile collection sample	C. Schnee
γ-cadinene	volatile collection sample	C. Schnee
limonene	(+)(-)-limonene	Sigma, (St. Louis, USA)
linalool	(R)(S)-linalool	Fluka
β-myrcene	β-myrcene	Fluka

In addition the Wiley library (Hewlett-Packard) and the NIST library (National Institute of Standards and Technology) were applied. In case no commercial source for the authentic standards was available, the compounds were compared with volatile collection samples of the same substances that were previously identified. The following GC columns were used: DB5-MS column ([5%-phenyl]-dimethylpolysiloxane, 30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W, Folsom, USA), DB-WAX column (polyethylene glycol, 30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W) and a Chrompack CP-SIL-5 Cb-MS column (dimethylpolysiloxane, 25 m x 0.25 mm i.d. x 0.25 µm film thickness, Varian, USA).

2.2.5. Parasitation rates

To examine the parasitation rates of lepidopterans in the field, an experimental setup according to Thaler (1999) was used: semitransparent boxes (diameter of 12 cm) without lid were positioned in the field within the canopy but without contacting the plants. When the plants reached a height over 1 m, the boxes were attached to the upper third of the plant (figure 2.6). 20 larvae of *S. littoralis* (third instar) were placed in the boxes and fed with leaf material of the corresponding cultivar and the leaf material was renewed every two days. The sidewalls of the containers were coated with teflon powder to avoid the escape of caterpillars. The boxes were covered with a metal mesh with a mesh size of approximately 2 cm, small enough to prevent birds from feeding on the larvae but big enough to enable parasitoids to enter the box. In the field near Halle, two boxes were positioned in each parcel of the lines tested for one week. This exposure was repeated once a month from June to September 2003. In Kitzingen, the experiments were conducted in July and August 2003. Here, Navares and Antares were tested with 4 boxes per parcel for two days. After exposition in the field, the caterpillars were reared on artificial diet in the laboratory until pupation to visualize possible parasitism.

Figure 2.6. Experimental setup for examination of parasitation rates

2.2.6. Statistical analysis

A two-factorial ANOVA was used to evaluate the data collected in the laboratory for effects of the transformation with Bt (factor 1) and feeding by different insect species (factor 2) as well as for interactions between those factors. This statistical test analyzes the data for significant differences within one factor after combining the data of the other factor. Also, interactions can be detected by this test when both factors are considered. Data were tested for normality and homogeneity of variances by the Kolmogorov-Smirnov-test (K-S-test) and Levenes test, respectively. To evaluate differences among the means of the treatment groups, Post Hoc tests were performed using Tukey's HSD test for equal variances and Dunnet's T3 test for unequal variances of data. The algebraic sign of the differences of means thereby indicates which group had the higher mean: positive data indicate a higher mean of the first mentioned group whereas negative data stand for a lower mean in the first mentioned group than in the second group. By using a χ^2-statistic, possible differences in the proportion of the volatiles in volatile blends from the different groups were analyzed (table 2.4) (SigmaStat 2.03 for Windows, 1992-1997).
To examine whether transformation with Bt, month, and year have an effect on the volatile composition of corn plants or whether there are interactions between those factors, the data collected in the field were analyzed using a three-factorial ANOVA for the pair of cultivars Prelude/Valmont and a two-factorial ANOVA for Nobilis/Novelis and Antares/Navares (see table 2.4). By using Kolmogorov-Smirnov-test (K-S-test) and Levenes test the data were tested for normality and homogeneity of variances, respectively. Post Hoc tests were performed to compare months and years by using Tukey's HSD test for equal variances and Dunnet's T3 test for unequal variances of data. The analysis was conducted by the GLM procedure of SPSS (SPSS 13.0 for Windows 2004). To examine and analyze differences in the proportions of individual volatiles in volatile blends of different groups, a χ^2-statistic was used (table 2.4) (SigmaStat 2.03 for Windows, 1992-1997). Graphs were created with the program SigmaPlot 7.0 (SPSS Inc.), showing arithmetic means and standard error if not indicated otherwise. By using Microsoft Excel 2002 SP3, pie charts were created showing the proportions of individual compounds within the volatile profile.

Table 2.4. Scheme of the volatile collections of the different corn lines in both fields
Pairs of cultivars: NN: Nobilis/ Novelis; PV: Prelude/ Valmont; AN: Antares/ Navares. Each pair of cultivar was tested separately for differences between Bt (transgenic) and non-Bt (isogenic), and additionally between years and months. Yellow marked characters represent data sets tested for interactions of Bt and month, and red characters represent data sets tested for interactions of Bt and year. [NN*]: this data set was not included in statistical analysis.

year	Field near Halle				Field near Kitzingen		
	June	July	August		July	August	
2001	NN	NN	NN		no data	no data	
2002	PV	PV	PV	NN	no data	AN	[NN*]
2003	PV	PV	PV	NN	AN	AN	

2.3. Results

2.3.1. Plant volatile analysis in the laboratory

2.3.1.1. Comparison between Nobilis (isogenic) and Novelis (transgenic)

To compare the composition of emitted volatiles between the transgenic line Novelis and the isogenic line Nobilis and its change after herbivore-damage, the odor emitted by both lines was collected from control plants that were left undamaged and treated plants after overnight-infestation with larvae of *S. littoralis*.
The volatile profiles in the laboratory experiments consisted of the green leaf volatile (*Z*)-3-hexen-1-yl acetate, the monoterpenes limonene, (*E*)-β-ocimene, and linalool, the homoterpene dimethyl nonatriene (DMNT) and the sesquiterpenes α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β- and (*E,E*)-α-farnesene, γ- and δ-cadinene, and β-sesquiphellandrene (table 2.5). The average release and standard deviations of the volatiles are shown in table A 26 in the appendix.

Factor Bt-transformation. The comparison of the transgenic and isogenic corn lines showed regardless of the various treatments significant differences in the release of limonene, (*E*)-β-ocimene, (*E*)-β-caryophyllene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, and the total amount of volatiles with a higher release in the isogenic line relative to the transgenic line (table 2.5). Linalool, otherwise, was emitted by the isogenic corn line in significantly decreased amounts. Furthermore, significant qualitative differences were found: α-ylangene and β-sesquiphellandrene could only be detected in the isogenic line Nobilis (tables 2.5 and A 26 and figure 2.7).

Factor treatment. Significant differences between the treatments (control vs *Spodoptera*-infestation) were found for all volatiles except for β-sesquiphellandrene and δ-cadinene with considerably enhanced amounts emitted by the infested plants, independently whether the plants were transformed with a Bt-coding gene or not (table 2.5).

Interactions between Bt-transformation and treatments. Both factors together, the transformation with a Bt-coding gene as well as the different treatments play an important role regarding their influence on the volatile profile. These interactions were significant for the volatiles limonene, (*E*)-β-ocimene, linalool, α-ylangene, (*E*)-β-caryophyllene, (*E*)-β- and (*E,E*)-α-farnesene, γ-cadinene, and the total amount of volatiles (table 2.5 and figure 2.7).

Table 2.5. Results of the Two-factorial Analysis of Variance for the effects of feeding by *Spodoptera littoralis*, transformation with Bt, and first-order interactions on the volatile emission in the corn lines Nobilis (isogenic) and Novelis (transgenic) in the laboratory
F-values and p-values are shown for differences in treatments, Bt, and interactions. Treatment: control versus feeding by Spodoptera littoralis; interaction: interaction of treatment and Bt; n.s.: not significant ($p>0.05$); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		treatment (dF=1)		interaction	
	F	p	F	p	F	p
(*Z*)-3-hexen-1-yl acetate	0.374	n.s.	21.798	0.000	1.182	n.s.
limonene	7.956	0.011	16.621	0.000	19.515	0.000
(E)-β-ocimene	11.330	0.003	39.028	0.000	27.196	0.000
linalool	16.470	0.001	30.527	0.000	7.501	0.013
DMNT	2.115	n.s.	26.332	0.000	1.487	n.s.
α-ylangene	5.179	0.010	7.219	0.014	7.219	0.014
(E)-β-caryophyllene	15.081	0.001	65.745	0.000	12.604	0.002
(E)-α-bergamotene	1.303	n.s.	13.038	0.002	0.439	n.s.
(E)-β-farnesene	24.430	0.000	34.525	0.000	11.046	0.003
(E,E)-α-farnesene	9.298	0.006	31.404	0.000	7.429	0.013
γ-cadinene	2.867	n.s.	9.754	0.005	7.075	0.015
δ-cadinene	0.079	n.s.	1.450	n.s.	0.157	n.s.
β-sesquiphellandrene	11.155	0.003	1.406	n.s.	1.406	n.s.
total volatiles	5.664	0.027	69.256	0.000	5.273	0.033

Although the total amount of emitted volatiles showed no differences between the isogenic and transgenic corn line in the undamaged control plants, differences in the release of individual volatiles occured (figure 2.7). The terpenoids limonene, β-ocimene, linalool, and γ-cadinene, for instance, were emitted in significantly higher amounts by the transgenic line than by the isogenic line with the highest increase at 30-fold for (*E*)-β-ocimene, whereas (*E*)-β-farnesene was significantly decreased to 15 % in the transgenic corn line compared to the isogenic corn line (figure 2.7 and table A 26). The sesquiterpenes (*E,E*)-α-farnesene and (*E*)-β-caryophyllene otherwise were released in equal amounts by both corn lines, and neither α-ylangene nor β-sesquiphellandrene could be detected in the transgenic line (figure 2.7 and table A 26). Hence, the proportions of the individual volatiles within the volatile profiles showed clear differences between uninfested transgenic and isogenic plants.
When plants of both corn lines were infested by *Spodoptera*-larvae, a different picture appeared. The isogenic line emitted a significantly higher amount of total volatiles, which was increased by 65 % in comparison to the transgenic line (figure 2.7 and table A 26). Also most volatiles such as limonene, (*E*)-β-ocimene, (*E*)-β-caryophyllene, (*E*)-β-farnesene, and (*E,E*)-α-farnesene were emitted in elevated amounts by the isogenic corn line when compared to the transgenic line (figure 2.7). The most dramatic difference was found for limonene showing a 6.5-fold higher emission by non-Bt-plants in comparison to the Bt-plants. While only the terpenoid alcohol linalool was released in significantly higher amounts by the transgenic line relative to the isogenic line, the other volatiles including γ-cadinene were emitted in equal amounts by both corn lines after herbivory. In the herbivory-treated transgenic plants, also no α-ylangene and β-sesquiphellandrene were found. As a result the proportions of the individual volatiles within the total volatile composition showed remarkable differences between the two corn lines for this treatment. By combining these results it could be demonstrated that non-transformed and Bt-transformed plants respond differently to *Spodoptera*-infestation. While the herbivore-

damaged isogenic line released higher amounts for single volatiles – 210-fold in case of (*E*)-β-ocimene – compared to their control plants, the increase in volatiles of herbivory-induced plants of the transgenic corn line was by far less pronounced relative to the corresponding control (figure 2.7 and table A 26). Here, the increase in emission by the herbivory-treated transgenic plants ranged from 2-times for (*Z*)-3-hexen-1-yl acetate to 15-times for (*E*)-β-caryophyllene relative to the untreated plants.

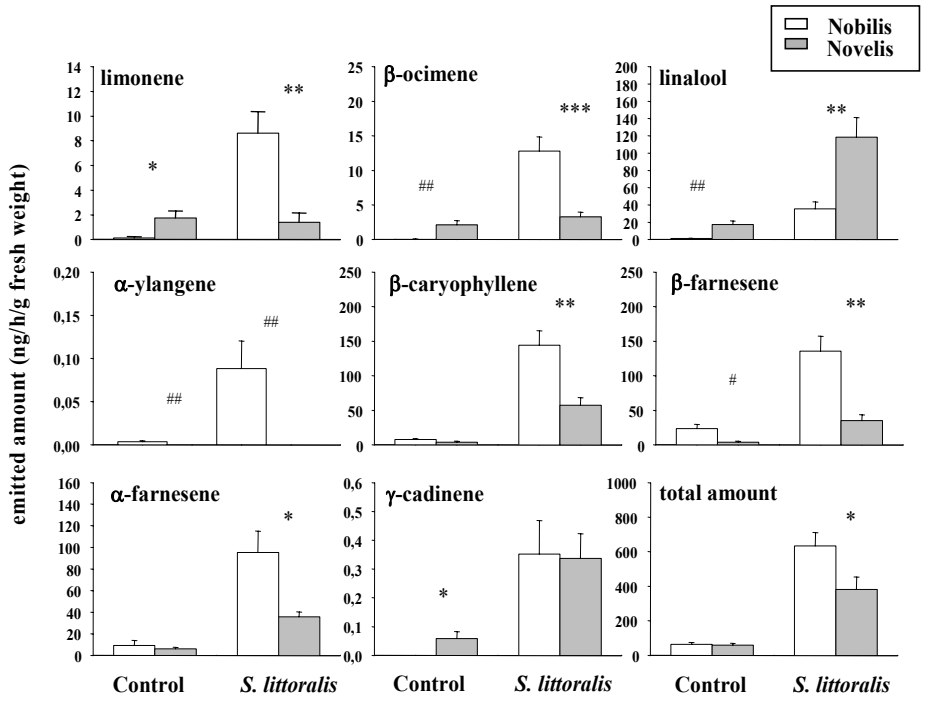

Figure 2.7. Emitted volatiles showing interactions between treatment and transformation with the Bt-toxin under larval feeding of *Spodoptera littoralis* on the lines Nobilis (isogenic) and Novelis (transgenic)
Stars (t-test) and double crosses (U-test) indicate significant differences between the lines within the same treatement (*/ $^{\#}$: $p<0,05$; **/ $^{\#\#}$: $p<0,01$; ***/ $^{\#\#\#}$: $p<0,001$); N = 6.

Proportions of the individual components within the volatile blend. The proportions of the components in the volatile mixtures showed highly significant differences for the comparison between transgenic and isogenic corn line within each treatment, but also for the comparison between control and herbivore-damage within each cultivar ($p<0.001$). Interestingly, the differences between the isogenic and transgenic lines within both the control and the *Spodoptera*-infested groups were more pronounced (χ^2-values 85.40 and 28.65, respectively) than the differences between control and herbivory-damage within Nobilis or Novelis (χ^2-values 20.74 and 15.37, respectively).

2.3.1.2. Comparison between Prelude (isogenic) and Valmont (transgenic)

In these experiments, plants of the transgenic line Valmont and the isogenic line Prelude were left untreated or infested with one of three different herbivore species: *Spodoptera littoralis, Ostrinia nubilalis*, or *Agrotis segetum*. The volatile blend of the herbivore-damaged plants and of the control plants was collected and analyzed for differences between the lines and the treatments.
The volatile profile in these experiments included (*Z*)-3-hexen-1-yl acetate, limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-α-

bergamotene, (*E*)-β- and (*E,E*)-α-farnesene, γ- and δ-cadinene, β-bisabolene, and β-sesquiphellandrene. In none of the treatments there were qualitative differences between the isogenic and transgenic corn lines (table 2.6). The average release and standard deviations of the volatiles are shown in table A 27 in the appendix.

Factor Bt-transformation. The comparison of the volatile profile showed irrespective of the treatments significantly lower amounts of (*E*)-β-ocimene, linalool, DMNT, α-ylangene, cycloisosativene, (*E,E*)-α-farnesene, γ-cadinene, and δ-cadinene as well as the total amount of volatiles released by the isogenic line relative to the transgenic line (tables 2.6 and A 27, and figure 2.8).

Factor treatment. Independently of the transformation with Bt, significant differences between treatments were found for the total amount of volatiles as well for all volatiles except for limonene (table 2.6).

Table 2.6. Results of the Two-factorial Analysis of Variance for the effects of feeding by different insect species, transformation with Bt, and first-order interactions on the volatile emission in the corn lines Prelude (isogenic) and Valmont (transgenic) in the laboratory
F-values and p-values are shown for differences in treatments, Bt, and interactions. Treatment: control versus larval feeding by the insect species *Spodoptera littoralis*, *Agrotis segetum*, or *Ostrinia nubilalis*; interaction: interaction of treatment and Bt; n.s.: not significant (p>0.05); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		treatment (dF=3)		interaction	
	F	p	F	p	F	p
(*Z*)-3-hexen-1-yl acetate	0.911	n.s.	99.767	0.000	1.409	n.s.
limonene	1.303	n.s.	1.494	n.s.	1.024	n.s.
(E)-β-ocimene	11.488	0.001	112.299	0.000	12.487	0.000
linalool	4.165	0.047	63.014	0.000	2.230	n.s.
DMNT	15.403	0.000	165.753	0.000	13.123	0.000
α-ylangene	10.678	0.002	73.301	0.000	3.472	0.023
cycloisosativene	14.705	0.000	85.077	0.000	14.808	0.000
(*E*)-β-caryophyllene	0.379	n.s.	16.316	0.000	15.524	0.000
(*E*)-α-bergamotene	1.783	n.s.	127.975	0.000	1.561	n.s.
(*E*)-β-farnesene	2.301	n.s.	127.400	0.000	2.091	n.s.
(*E,E*)-α-farnesene	6.268	0.016	66.861	0.000	3.698	0.018
γ-cadinene	10.860	0.002	82.213	0.000	3.857	0.015
δ-cadinene	74.852	0.000	70.969	0.000	65.278	0.000
β-bisabolene	1.053	n.s.	124.969	0.000	0.905	n.s.
β-sesquiphellandrene	0.571	n.s.	115.191	0.000	0.541	n.s.
total volatiles	5.360	0.025	165.819	0.000	3.552	0.021

When comparing the volatile blend of the corn plants after exposure to three different herbivores, no consistent pattern in the emission was detectable. Uninfested plants emitted (*Z*)-3-hexen-1-yl acetate, limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-β- and (*E,E*)-α-farnesene, and γ- and δ-cadinene.
The volatile profile of plants exposed to *Spodoptera*-feeding also included these volatiles but in contrast to the control plants, no cycloisosativene was detected (table A 27). As shown in table 2.7 after *Spodoptera*-infestation the plants emitted significantly elevated amounts of most individual volatiles in comparison to the control except for δ-cadinene and (*E*)-β-caryophyllene. Also, the total volatiles increased significantly to ~ 30-fold of that emitted by control plants (tables 2.7 and A 27). The most dramatic increase in emission was found for the terpenoid alcohol linalool and the sesquiterpene (*E*)-β-

farnesene which were emitted in up to 130- and 600-fold higher amounts relative to the corresponding controls.
When the plants were exposed to feeding by *Ostrinia nubilalis* they released a similar volatile profile like the control plants, but in contrast to the control and all other treatments, no δ-cadinene was found (table A 27). Significant differences between the release of control plants and *Ostrinia*-damaged plants were found for the total volatiles as well as for most individual volatiles except for (*E*)-β-ocimene, (*E*)-β-caryophyllene, (*E,E*)-α-farnesene, and β-bisabolene (table 2.7). Most individual volatiles and the total amount were released in higher rates after exposure to *O. nubilalis* relative to the control, whereas some terpenes like limonene, cyclosisosativene, or (*E,E*)-α-farnesene showed a higher release in the control plants.

Table 2.7. Effects of feeding by different insect species on the volatile emission in the corn lines Prelude (isogenic) and Valmont (transgenic) in the laboratory
Post hoc test (Dunnet's T3) showed differences in mean volatile emission (ng/h/g fresh weight) and p-values. Negative diff. of means indicate higher amounts of the treatment within each comparison, while positive diff. of means represent higher amounts of the control. n.s.: not significant (p>0.05); N = 6.

volatile compound	control vs *S. littoralis*		control vs *O. nubilalis*		control vs *A. segetum*	
	diff. means	p	diff. means	p	diff. means	p
(*Z*)-3-hexen-1-yl acetate	-23.342	0.000	-0.646	0.006	-2.155	n.s.
limonene	-0.272	0.000	0.360	0.008	-0.439	n.s.
(E)-β-ocimene	-5.910	0.001	0.206	n.s.	-0.192	n.s.
linalool	-125.370	0.000	-1.771	0.007	-13.772	n.s.
DMNT	-53.380	0.000	-1.262	0.006	-1.699	n.s.
α-ylangene	-0.021	0.001	0.004	0.001	-0.007	0.000
cycloisosativene	1.932	0.000	1.678	0.001	1.933	0.000
(*E*)-β-caryophyllene	-2.115	n.s.	-0.438	n.s.	0.153	n.s.
(*E*)-α-bergamotene	-87.303	0.000	-2.404	0.000	-2.215	n.s.
(*E*)-β-farnesene	-245.584	0.000	-6.876	0.000	-4.508	n.s.
(*E,E*)-α-farnesene	-52.064	0.001	1.718	n.s.	-7.275	0.034
γ-cadinene	-0.050	0.000	-0.017	0.013	-0.010	n.s.
δ-cadinene	0.567	n.s.	0.651	0.041	0.630	0.048
β-bisabolene	-5.536	0.000	0.000	n.s.	0.000	n.s.
β-sesquiphellandrene	-19.210	0.000	-0.570	0.000	-0.421	n.s.
total amount	-618.110	0.000	-9.662	0.014	-29.976	n.s.

After damage by *Agrotis segetum* most volatiles were present as in the control plants, but similar to *Spodoptera*-infestation, no release of cycloisosativene and furthermore no β-bisabolene was found. The comparison of the volatiles released by control plants and plants infested with *A. segetum* showed significant differences only for some terpenes: α-ylangene and (*E,E*)-α-farnesene were released in higher amounts relative to control while δ-cadinene was released in lower amounts and cycloisosativene could not be detected in *Agrotis*-damaged plants (tables 2.7 and A 27).
When comparing the release of volatiles induced by *S. littoralis* and *O. nubilalis*, significant differences were found for the total amount and all individual volatiles except for (*E*)-β-caryophyllene (table 2.8). The damage by *S. littoralis* led generally to a higher release of the total amount and individual volatiles compared to feeding by *O. nubilalis* (tables 2.8 and A 27 and figures 2.8 and 2.9). The only exception was cycloisosativene which was found to be emitted only by the *Ostrinia*-infested plants. A similar result could be shown for the comparison between infestations by *Agrotis segetum* and *Spodoptera*

littoralis. Here, the plants that were damaged by *S. littoralis* also emitted significantly higher amounts of total volatiles and individual volatiles except for cycloisosativene and limonene (tables 2.8 and A 27 and figures 2.8 and 2.9). The comparison between *Ostrinia*-infestation and exposure to *A. segetum* showed significant differences only for some sesquiterpenes (table 2.8). While the plants damaged by *O. nubilalis* emitted significantly lower amounts of α-ylangene, (*E,E*)-α-farnesene, γ-cadinene, and δ-cadinene than that infested by *A. segetum*, the sesquiterpene cycloisosativene was only detectable after *Ostrinia*-feeding. These results illustrate that the corn plants responded differently to infestation by three herbivore-species both with qualitative and quantitative changes in the volatile composition thus altering the proportions of the individual volatiles between the treatments (see also figure 2.9 and table 2.8).

Table 2.8. Effects of feeding by different insect species on the volatile emission in the corn lines Prelude (isogenic) and Valmont (transgenic) in the laboratory
Post hoc test (Dunnet's T3) showed differences in mean volatile emission (ng/h/g fresh weight) and p-values. Negative diff. of means indicate higher amounts of the second treatment within each comparison, while positive diff. of means represent higher amounts of the first treatment. n.s.: not significant (p>0.05); N = 6.

volatile compound	***S. littoralis* vs *O. nubilalis***		***S. littoralis* vs *A. segetum***		***O. nubilalis* vs *A. segetum***	
	diff. means	**p**	**diff. means**	**p**	**diff. means**	**p**
(*Z*)-3-hexen-1-yl acetate	22.697	0.000	21.189	0.000	-1.508	n.s.
limonene	0.308	0.000	-0.167	n.s.	-0.475	n.s.
(*E*)-β-ocimene	6.116	0.000	5.718	0.001	-0.398	n.s.
linalool	123.599	0.001	111.598	0.001	-12.001	n.s.
DMNT	52.119	0.000	51.681	0.000	-0.438	n.s.
α-ylangene	0.251	0.000	0.144	0.036	-0.106	0.000
cycloisosativene	-0.255	0.000	0.000	n.s.	0.255	0.000
(*E*)-β-caryophyllene	1.677	n.s.	2.268	0.048	0.591	n.s.
(*E*)-α-bergamotene	84.898	0.000	85.088	0.000	0.189	n.s.
(*E*)-β-farnesene	238.708	0.000	241.077	0.000	2.369	n.s.
(*E,E*)-α-farnesene	53.782	0.001	44.789	0.002	-8.993	0.007
γ-cadinene	0.066	0.000	0.040	0.001	-0.026	0.000
δ-cadinene	0.084	0.002	0.064	0.010	-0.021	0.001
β-bisabolene	5.536	0.000	5.536	0.000	0.000	n.s.
β-sesquiphellandrene	18.640	0.000	18.790	0.000	0.149	n.s.
total amount	608.448	0.000	588.134	0.000	-20.315	n.s.

Interactions between Bt-transformation and treatments. Both factors together, the transformation with Bt as well as the various treatments, considerably influenced the volatile composition of the corn plants. These interactions were found to be significant for (*E*)-β-ocimene, DMNT, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E,E*)-α-farnesene, γ- and δ-cadinene and the total amount of volatiles (table 2.6 and figure 2.8). In general, most volatiles were emitted in higher amounts by the transgenic line than by the isogenic line or showed no differences between the corn lines, independently whether the plants were previously exposed to herbivory or left untreated. However, when comparing the volatile profile of both cultivars, some individual volatiles showed an inconsistent pattern in up- or down regulation among the treatments.
In the group of control plants, for instance, the total amount emitted as well as most volatiles were found to be emitted in equal amounts in both lines (table A 27 and figure 2.8). However, some volatiles showed a significant higher emission in the transgenic corn line such as the sesquiterpenes α-ylangene, cycloisosativene, γ-cadinene, and δ-cadinene.

The most dramatic increase in emission was found for δ-cadinene, which was released by the Bt-plants in a 30-times higher amount than in the non-Bt-plants (table A 27). After infestation by *Spodoptera*-larvae many volatiles showed comparable emissions between the maize cultivars (table A 27). The terpenes β-ocimene, DMNT, and δ-cadinene, otherwise, were significantly increased by 50 to 100 % in the Bt-plants relative to the non-Bt-plants (figure 2.8). Although the differences for the terpenes α-ylangene, (*E,E*)-α-farnesene, γ-cadinene and the total volatiles were not significant (figure 2.8) the release showed a similar trend. A contrary picture was found for the herbivory-induced sesquiterpene (*E*)-β-caryophyllene, which was released by plants of the transgenic line after exposure to *S. littoralis* in significantly lower amounts than by plants of the isogenic corn line (figure 2.8).

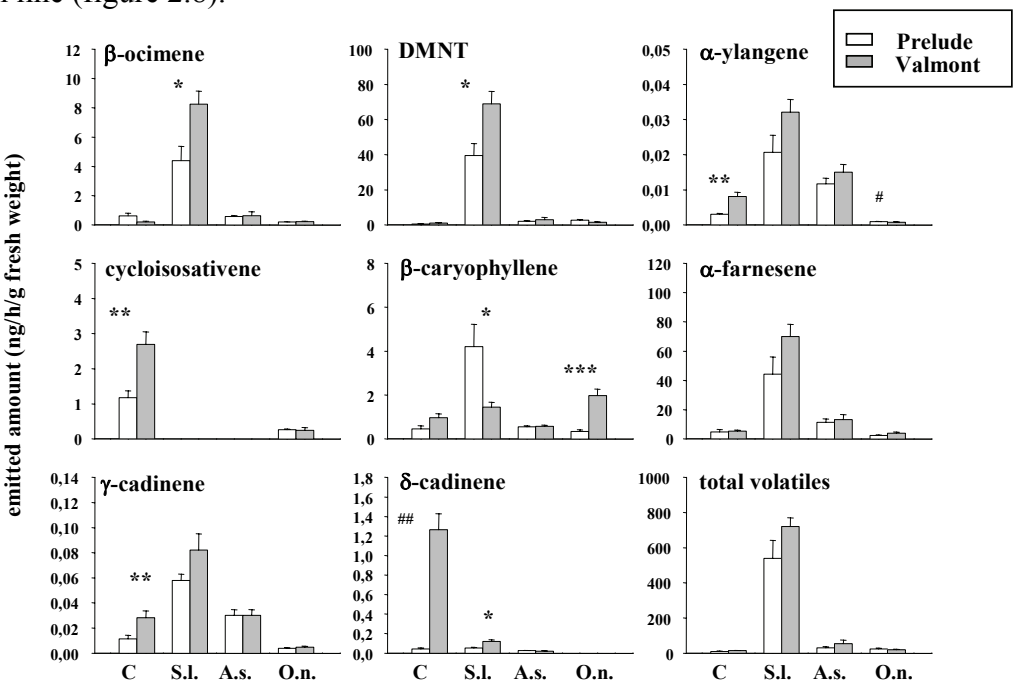

Figure 2.8. Emitted volatiles showing interactions between treatment and transformation with Bt under different larval feeding on the lines Prelude (isogenic) and Valmont (transgenic)

C: control; S.l.: Spodoptera littoralis; A.s.: Agrotis segetum; O.n.: Ostrinia nubilalis. Stars (t-test) and double crosses (U-test) indicate significant differences between the lines within the same treatment (*/ #: p<0,05; **/ ##: p<0,01; ***/ ###: p<0,001); N = 6.

After *Agrotis*-infestation most volatiles were released in equal amounts by both cultivars. However, limonene, linalool, (*E*)-α-bergamotene, (*E,E*)-β-farnesene, and β-sesquiphellandrene as well as the total amount of volatiles showed in a trend to be released in elevated amounts by the transgenic plants relative to the isogenic line (table A 27), although these differences were not significant. When the plants were treated with *Ostrinia*-feeding, differences in emission by the transgenic line in comparison to the isogenic line were found only for the herbivory-induced sesquiterpene (*E*)-β-caryophyllene which was increased by factor five while all other volatiles were released in equal amounts by both corn lines (table A 27 and figure 2.8).

Consequently, damage by the three different herbivores influenced the volatile emission of the transgenic and isogenic cultivars differently depending on the feeding herbivore. Whereas many volatile compounds were emitted in equal amounts, others for example the

herbivory-induced sesquiterpene (*E*)-β-caryophyllene or DMNT and α-ylangene showed an inconsistent pattern in emission among all treatments and between the lines (figure 2.8).

Proportions of the individual components within the volatile blend. As illustrated in table 2.9 and figure 2.9, the proportions of the individual components within the volatile profile showed highly significant differences between the corn lines as well as between the damage by the different herbivores. Remarkably, the differences were greater between the treatments within each corn line than between the corn lines within each treatment (table 2.9 and figure 2.9). Whereas the volatile profile of plants exposed to feeding by *A. segetum* consisted of up to 50 % mono- and homoterpenes and green leaf volatiles, the volatile blends of those plants which were either treated with *O. nubilalis* or *S. littoralis* and of the controls were mainly composed of sesquiterpenes. There were also differences in the composition of the sesquiterpene-fractions between the different treatments. Whereas the main sesquiterpene of both the controls and *Agrotis*-infested plants was (*E,E*)-α-farnesene with up to 42 % of total, the plants which were either treated with *O. nubilalis* or *S. littoralis* emitted as the major sesquiterpene (*E*)-β-farnesene with up to 40 %.

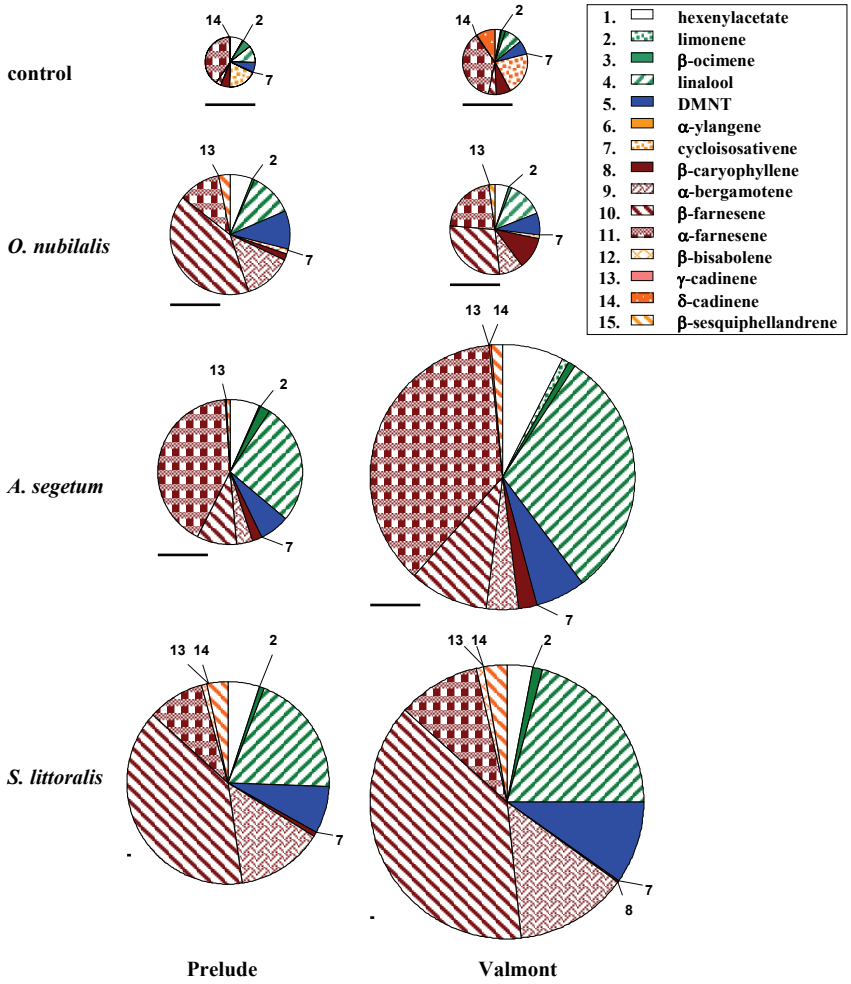

Figure 2.9. Comparison of the volatile profile after feeding by different herbivore species on the corn cultivars Prelude (isogenic) and Valmont (transgenic)

The mean percentages are depicted for the individual volatiles. White represents the green leaf volatiles, green colours the monoterpenes, blue colours the homoterpenes, red colours the sesquiterpenes induced by herbivory, and orange colours the remaining sesquiterpenes. The line beneath each circle corresponds to an amount of 10 ng and the diameter of each circle corresponds to the total amount emitted (ng/h/g fresh weight). Numbers indicate volatiles that were released in amounts below 1.5 % of the total emission.

Table 2.9. Comparison of the volatile composition after feeding by different insect species on the corn cultivars Prelude (isogenic) and Valmont (transgenic)
Results of the χ^2-statistics are shown for the comparison between the feeding by different herbivores within the pair of cultivar as well as for the comparison between the feeding by different herbivores for each cultivar. Prel: Prelude; Val: Valmont; *S. littoralis*: feeding by *Spodoptera littoralis*; *O. nubilalis*: feeding by *Ostrinia nubilalis*; *A. segetum*: Feeding by *Agrotis segetum*; n.t.: not tested. The numbers above the diagonal line represent the p-values, and the numbers below the diagonal line represent the χ^2-values.

p / χ^2		control		*S. littoralis*		*O. nubilalis*		*A. segetum*	
		Prel	**Val**	**Prel**	**Val**	**Prel**	**Val**	**Prel**	**Val**
control	**Prel**		<0.001	<0.001	n.t.	<0.001	n.t.	<0.001	n.t.
	Val	13.31		n.t.	<0.001	n.t.	<0.001	n.t.	<0.001
S. littoralis	**Prel**	97.69	n.t.		n.t.	<0.001	n.t.	<0.001	n.t.
	Val	n.t.	104.43	1.27		<0.001	<0.001	n.t.	<0.001
O. nubilalis	**Prel**	35.94	n.t.	50.92	n.t.		n.t.	<0.001	n.t.
	Val	n.t.	54.8	n.t.	46.19	1.32		n.t.	<0.001
A. segetum	**Prel**	86.91	n.t.	5.42	n.t.	53.71	n.t.		<0.001
	Val	n.t.	63.11	n.t.	22.55	n.t.	30.47	14.67	

2.3.1.3. Comparison between Antares (isogenic) and Navares (transgenic)

To examine the effect of transformation with Bt within the pair of cultivars Antares/ Navares, the odor emitted by the plants after infestation by either *S. littoralis* or *O. nubilalis* or by uninfested control plants was collected and compared.
Qualitative differences between transgenic and isogenic corn line were found neither in the control plants nor in the herbivory-treatments (table A 28). As illustrated in table 2.10 the volatile profile included β-myrcene, limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, γ-cadinene, and δ-cadinene. The average release and standard deviations of the volatiles are shown in table A 28 in the appendix.

Factor Bt-transformation. Significant differences between the transgenic line and the isogenic line were found independently of the various treatments for limonene, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-β-farnesene, and δ-cadinene which were released in higher amounts by the transgenic cultivar (table 2.10).

Factor treatment. Irrespective of the transformation of the plants with a Bt-coding gene there were significant differences between the treatments found for all individual terpenes but not for the total amount of emission (table 2.10). The volatile blend emitted by the control plants was composed of linalool, DMNT, α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, and (*E*)-β-farnesene (table 2.10 and A 28). Plants exposed to herbivory by *S. littoralis*, on the other hand, emitted additionally to these volatiles β-myrcene, limonene, (*E*)-β-ocimene, cycloisosativene, (*E,E*)-α-farnesene, and γ- and δ-cadinene. As demonstrated in table 2.11, the emission of each single compound and the total amount of volatiles was significantly elevated after damage by larvae of *S. littoralis* compared to the control plants with the most dramatic increase for linalool and (*E*)-β-caryophyllene (table A 28 and figure 2.10). After damage by *O. nubilalis* the same volatiles were found as released by the control plants except for α-ylangene, but additionally (*E,E*)-α-farnesene, and γ- and δ-cadinene were detected. Most individual volatiles as well as the total amount were released in elevated amounts by *Ostrinia*-infested plants compared to the control plants although these differences were only significant for limonene, γ- and δ-cadinene, and the total amount (table 2.11). The emission of the sesquiterpene α-ylangene also showed significant differences between

control and *Ostrinia*-infested plants such that it was emitted by the control plants but not after herbivory by *O. nubilalis* (table A 28). Significant differences between *Spodoptera*-feeding and exposure to *O. nubilalis* were found for most terpenes, showing higher emissions in the plants that were induced by *S. littoralis* (tables A 28 and 2.11). The only exceptions were (*E*)-α-bergamotene that was emitted in equal amounts by the plants after both herbivory-treatments, and the total amount of volatiles (table 2.11). Additionally, the terpenes β-myrcene, α-ylangene and cycloisosativene were only released after *Spodoptera*-infestation, but not after damage by *Ostrinia nubilalis* (table A 28).

Table 2.10. Results of the Two-factorial Analysis of Variance for the effects of feeding by different insect species, transformation with Bt, and first-order interactions on the volatile emission in the corn lines Antares (isogenic) and Navares (transgenic) in the laboratory

F-values and p-values are shown for differences in treatments, Bt, and interactions. Treatment: control versus larval feeding by the insect species *Spodoptera littoralis* and *Ostrinia nubilalis*; interaction: interaction of treatment and Bt; n.s.: not siginificant (p>0.05); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		treatment (dF=2)		interaction	
	F	p	F	p	F	p
β-myrcene	2.572	n.s.	99.268	0.000	2.727	n.s.
limonene	6.283	0.017	42.499	0.000	4.166	0.023
(*E*)-β-ocimene	1.602	n.s.	42.405	0.000	1.699	n.s.
linalool	0.476	n.s.	50.464	0.000	0.289	n.s.
DMNT	0.044	n.s.	31.117	0.000	0.064	n.s.
α-ylangene	8.961	0.005	30.609	0.000	9.172	0.001
cycloisosativene	8.065	0.007	27.738	0.000	8.554	0.001
(*E*)-β-caryophyllene	7.470	0.009	42.687	0.000	9.800	0.000
(*E*)-α-bergamotene	0.338	n.s.	6.062	0.005	3.799	0.031
(*E*)-β-farnesene	10.838	0.002	54.019	0.000	7.635	0.002
(*E,E*)-α-farnesene	0.346	n.s.	25.642	0.000	0.318	n.s.
γ-cadinene	3.610	n.s.	53.377	0.000	6.264	0.004
δ-cadinene	8.260	0.007	25.394	0.000	9.056	0.001
total volatiles	0.901	n.s.	0.660	n.s.	0.803	n.s.

Table 2.11. Effects of feeding by different insect species on the volatile emission in the corn lines Antares (isogenic) and Navares (transgenic) in the laboratory

Post hoc test (Dunnet's T3) showed differences in mean volatile emission (ng/h/g fresh weight) and p-values. Negative diff. means indicate higher amounts of the second treatment within each comparison, while positive diff. means represent higher amounts of the first treatment. n.s.: not significant (p>0.05); N = 6.

volatile compound	control vs *S. littoralis*		control vs *O. nubilalis*		*S. littoralis* vs *O. nubilalis*	
	diff. means	p	diff. means	p	diff. means	p
β-myrcene	-0.513	0.000	0.000	n.s.	0.513	0.000
limonene	-2.520	0.000	-1.079	0.000	1.441	0.001
(*E*)-β-ocimene	-3.873	0.000	0.000	n.s.	3.873	0.000
linalool	-99.280	0.000	-4.045	n.s.	95.235	0.000
DMNT	-43.380	0.000	-0.469	n.s.	42.912	0.000
α-ylangene	-0.0429	0.001	0.001	0.001	0.044	0.001
cycloisosativene	-20.045	0.001	0.000	n.s.	20.045	0.001
(*E*)-β-caryophyllene	-32.700	0.000	-1.196	n.s.	31.504	0.000
(*E*)-α-bergamotene	-2.603	0.000	-1.517	n.s.	1.085	n.s.
(*E*)-β-farnesene	-12.305	0.000	-0.571	n.s.	11.733	0.000
(*E,E*)-α-farnesene	-25.688	0.000	-0.449	n.s.	25.240	0.000
γ-cadinene	-0.081	0.000	-0.009	0.000	0.715	0.000
δ-cadinene	-0.357	0.002	-0.012	0.000	0.346	0.002
total volatiles	-243.387	0.000	-232.818	0.000	-10.569	n.s.

Interactions between Bt-transformation and treatments. Both factors together, the transformation of the plants with a Bt-coding gene as well as the different treatments, altered the volatile composition of the plants. These interactions were found to be significant for the terpenes limonene, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, and γ- and δ-cadinene (table 2.10 and figure 2.10). When comparing the volatile blend of both corn lines after exposure to the different herbivores, incoherent emission patterns for the treatments as well as within the corn lines were detectable. Control plants of both lines emitted comparable amounts of all individual terpenes. When plants of both lines were exposed to feeding by larvae of *S. littoralis*, the emitted amounts of most terpenes including limonene, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, or δ-cadinene line were significantly increased by the transgenic up to 4-times compared to the emissions by the isogenic line (table A 28). The other terpenes and the total amount of volatiles were released in equal amounts by both lines. In the group that was treated with *Ostrinia*-infestation γ-cadinene showed a significant decrease in the transgenic line relative to the isogenic line, while all other terpenes and the total volatiles were released equally by both lines (table A 28 and figure 2.10). This led to the assumption that the rates of terpene emission were decreased in the isogenic line relative to the transgenic line after damage by *S. littoralis*, whereas these volatiles showed an equal or even higher emission by the isogenic line when the plants were infested by *O. nubilalis* (figure 2.10).

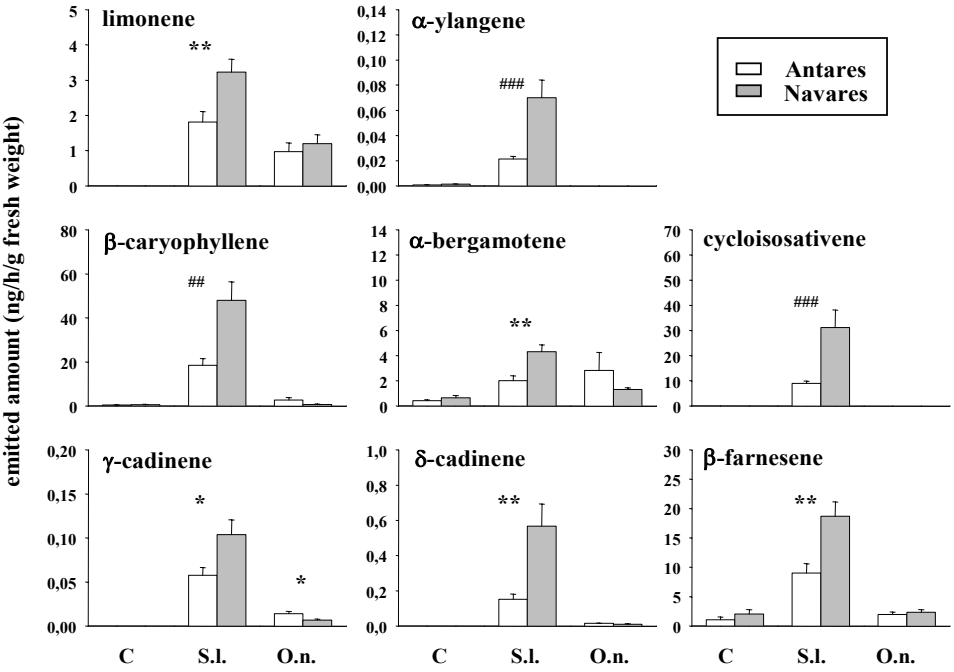

Figure 2.10. Emitted volatiles showing interactions between treatment and transformation with the Bt-toxin under different larval feeding on the lines Antares (isogenic) and Navares (transgenic)

C: control; S.l.: *Spodoptera littoralis*; O.n.: *Ostrinia nubilalis*. Stars (t-test) and double crosses (U-test) indicate significant differences between the lines within the same treatment (*/ #: p<0,05; **/ ##: p<0,01; ***/ ###: p<0,001); N = 6.

Proportions of the individual components within the volatile blend. As illustrated in table 2.12, there were highly significant differences between the proportions of the individual components in the volatile profile within each cultivar as well as between the

treatments. The differences between treatments and between cultivars were comparable indicating that treatment and cultivar equally influence the volatile composition.

Table 2.12. Comparison of the volatile composition after feeding by different insect species on the corn cultivars Navares (isogenic) and Antares (transgenic)
Results of the χ^2-statistics are shown for the comparison between the feeding by different herbivores within the pair of cultivar as well as for the comparison between the feeding by different herbivores for each cultivar. Nav: Navares; Ant: Antares; *S. littoralis*: feeding by *Spodoptera littoralis*; *O. nubilalis*: feeding by *Ostrinia nubilalis*; n.t.: not tested. The numbers above the diagonal line represent the p-values, and the numbers below the diagonal line represent the χ^2-values.

χ^2 \ p		control		*S. littoralis*		*O. nubilalis*	
		Nav	**Ant**	**Nav**	**Ant**	**Nav**	**Ant**
control	**Nav**		<0.001	<0.001	n.t.	<0.001	n.t.
	Ant	18.76		n.t.	<0.001	n.t.	<0.001
S. littoralis	**Nav**	48.56	n.t.		<0.001	<0.001	n.t.
	Ant	n.t.	46.23	7.82		n.t.	<0.001
O. nubilalis	**Nav**	13.65	n.t.	51.00	n.t.		<0.001
	Ant	n.t.	47.03	n.t.	89.38	90.74	

2.3.2. Plant volatile analysis in the field

To investigate possible qualitative or quantitative differences in the volatile blend of transgenic corn plants in comparison to the isogenic corn line under field conditions, three different pairs of cultivars were grown in two field sites and the volatiles were collected from June to August over the years 2001-2003 (table 2.4 and figures 2.3 and 2.4). Additionally, the volatile profile was analyzed for changes during a field season and between three consecutive years.

2.3.2.1. Comparison between the lines Nobilis (isogenic) and Novelis (transgenic)

- **The volatile blend within the field season 2001**

The volatiles of the transgenic corn line Novelis and the isogenic corn line Nobilis were analyzed over the course of a field season from June to August 2001 in the field near Halle (see table 2.4). In this year, the volatile profile was composed of limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β- and (*E,E*)-α-farnesene, and γ- and δ-cadinene (table 2.13). The average release and standard deviations of all compounds as well as of the total volatiles are shown in table A 29 in the appendix. The release of these compounds was examined with regard to a possible influence of the factors Bt-transformation and months.

Factor Bt-transformation. Independently of the months, significant differences between the transgenic and isogenic lines were found for limonene, α-ylangene and (*E*)-α-bergamotene (table 2.13). While limonene was released in elevated amounts by the transgenic cultivar relative to the isogenic line, the contrary trend was found for α-bergamotene. The sesquiterpene α-ylangene could only be detected in the isogenic corn line.

Factor month. After combining the data of both cultivars, significant differences amongst the months were found for the sesquiterpenes (*E*)-β-caryophyllene with higher amounts in June compared to July (Dunnet's T3 post hoc test: diff. means = 504.699 ng/h/plant, p = 0.024) and (*E,E*)-α-farnesene with elevated amounts in June relative to

August (Dunnet's T3 post hoc test: diff. means = 207.558 ng/h/plant, p = 0.015) (table 2.13).

Table 2.13. Results of the Two-factorial Analysis of Variance for the effects of transformation with Bt, months, and first-order interactions on the volatile emission in the corn lines Novelis (transgenic) and Nobilis (isogenic) in the field near Halle in 2001
F-values and p-values are shown for differences between the isogenic line Nobilis and the transgenic line Novelis, months, and interactions. month: June to August; n.s.: not siginificant (p>0.05); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		month (dF=2)		interaction (Bt x month)	
	F	p	F	p	F	p
limonene	18.370	0.000	2.314	n.s.	1.688	n.s.
(*E*)-β-ocimene	1.754	n.s.	0.983	n.s.	2.296	n.s.
linalool	1.164	n.s.	1.232	n.s.	3.548	0.041
DMNT	0.071	n.s.	0.257	n.s.	3.812	0.033
α-ylangene	41.566	0.000	1.539	n.s.	1.539	n.s.
(*E*)-β-caryophyllene	0.533	n.s.	4.020	0.028	2.229	n.s.
(*E*)-α-bergamotene	8.078	0.008	0.845	n.s.	1.925	n.s.
(*E*)-β-farnesene	0.259	n.s.	1.524	n.s.	5.273	0.011
(*E,E*)-α-farnesene	1.353	n.s.	4.568	0.019	0.320	n.s.
γ-cadinene	1.139	n.s.	1.198	n.s.	3.525	0.042
δ-cadinene	3.489	n.s.	0.088	n.s.	1.294	n.s.
total volatiles	0.038	n.s.	1.828	n.s.	2.519	n.s.

Interactions between Bt-transformation and month. Both factors together, the transformation with a Bt-coding gene as well the months, strongly influenced the release of some terpenes by the maize plants. These interactions were detected to be significant for linalool, DMNT, (*E*)-β-farnesene and γ-cadinene (table 2.13 and figure 2.11). Whereas the terpenes linalool, DMNT and γ-cadinene were released rather constantly over the field season by the isogenic line, the emission of these compounds was decreased from June to August in the transgenic corn line.

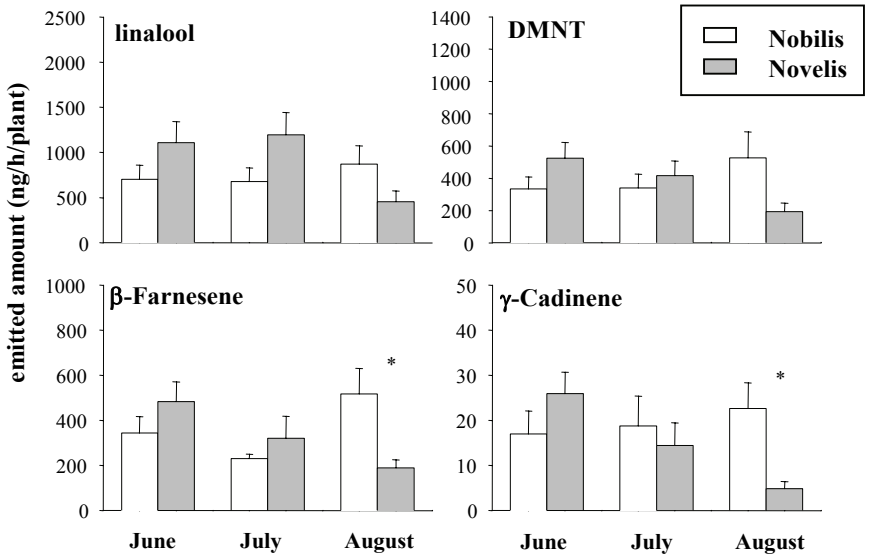

Figure 2.11. Emitted volatiles showing interactions between months and transformation with the Bt-toxin in the lines Nobilis (isogenic) and Novelis (transgenic) in the field near Halle 2001
Stars (t-test) indicate significant differences between the lines within the same treatment (*: p<0,05; **: p<0,01; ***: p<0,001); N = 6.

This trend was even more pronounced for the sesquiterpene (*E*)-β-farnesene since both cultivars showed opposite patterns in release. The emission of this volatile increased from June till August in the isogenic line while in the transgenic line the amount continuously decreased over the field season, thus resulting in significant differences between the lines in the month August (figure 2.11). However, the total amount of volatiles showed a comparable tendency for the transgenic cultivar as illustrated above for the individual compounds, since most compounds showed a decrease over the field season (table A 29).

Given these results, seven compounds within the volatile blend were influenced in their release by the transformation with a Bt-coding gene out of which three - limonene, α-ylangene, and (*E*)-α-bergamotene - showed differences between both corn lines over the complete field season and four - linalool, DMNT, (*E*)-β-farnesene, and γ-cadinene - were found to be influenced in certain months (table 2.13 and figure 2.11).

Proportions of the individual components within the volatile blend. As demonstrated in table 2.14, highly significant differences in the volatile composition between the corn lines within each month as well as between the months occured. Both, the comparison between the maize cultivars within each month as well as between the months within each cultivar showed comparable χ^2-values suggesting that both months and cultivar equally influenced the proportions of individual compounds within the volatile profile.

Table 2.14. Comparison of the volatile composition released by the corn cultivars Nobilis (isogenic) and Novelis (transgenic) in the field in 2001

Results of the χ^2-statistics are shown for the comparison of the volatile emission between the months in 2001 within the pair of cultivar as well as for each cultivar. n.t.: not tested. The numbers above the diagonal line represent the p-values, and the numbers below the diagonal line represent the χ^2-values.

χ^2 \ p		June		July		August	
		Nobilis	**Novelis**	**Nobilis**	**Novelis**	**Nobilis**	**Novelis**
June	**Nobilis**		<0.001	<0.001	n.t.	<0.001	n.t.
	Novelis	1.86		n.t.	<0.001	n.t.	<0.001
July	**Nobilis**	3.22	n.t.		<0.001	<0.001	n.t.
	Novelis	n.t.	5.92	18.33		n.t.	<0.001
August	**Nobilis**	5.45	n.t.	10.58	n.t.		<0.001
	Novelis	n.t.	5.73	n.t.	1.53	10.06	

- **The volatile blend in August among the years 2001 to 2003**

The volatiles of the transgenic corn line Novelis and the isogenic corn line Nobilis were analyzed in August of three consecutive years in the field near Halle (see table 2.4). The following volatiles were found: limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β- and (*E,E*)-α-farnesene, β- and α-selinene, β-bisabolene, and γ- and δ-cadinene (table 2.15). The average release and standard deviations of all individual volatiles and of the total amount are shown in tables A 29 and A 30 in the appendix. The release of these compounds was examined with respect to a possible influence of the factors Bt-transformation and years.

Factor Bt-transformation. After combining the data of the different years, there were significant differences between the isogenic and transgenic corn line found for the total amount emitted as well as for α-ylangene and γ-cadinene (table 2.15). The sesquiterpene α-ylangene was exclusively emitted by the isogenic corn line whereas the release of γ-cadinene was increased in the isogenic line by the factor five relative to the transgenic line

(table A 29). The total emission was increased in the isogenic cultivar relative to the transgenic cultivar.

Factor year. Regardless of the Bt-transformation, significant differences between years were found for the total volatile emission and for all terpenes except for limonene and (*E*)-β-ocimene (table 2.15). Hereby, qualitative differences in the composition of the volatile blend between all years appeared for the group of sesquiterpenes but not for mono- and homoterpenes. In 2001 the plants emitted the sesquiterpenes α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, and γ- and δ-cadinene. In 2002, the following season, two new compounds, β- and α-selinene, were detected instead of the sesquiterpenes released in 2001 (tables A 29 and A 30).

Table 2.15. Results of the Two-factorial Analysis of Variance for the effects of transformation with Bt, years, and first-order interactions on the volatile emission in the corn lines Novelis (transgenic) and Nobilis (isogenic) in the field near Halle
F-values and p-values are shown for the differences between the isogenic line Nobilis and the transgenic line Novelis, years, and interactions. year: 2001 to 2003; n.s.: not siginificant (p>0.05); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		year (dF=2)		interaction (Bt x year)	
	F	p	F	p	F	p
limonene	2.581	n.s.	0.120	n.s.	1.027	n.s.
(*E*)-β-ocimene	0.431	n.s.	1.121	n.s.	1.727	n.s.
linalool	3.286	n.s.	4.770	0.016	1.971	n.s.
DMNT	1.104	n.s.	15.740	0.000	7.299	0.003
α-ylangene	11.924	0.002	9.339	0.001	9.339	0.001
(*E*)-β-caryophyllene	2.591	n.s.	21.523	0.000	2.591	n.s.
(*E*)-α-bergamotene	3.386	n.s.	8.141	0.001	2.035	n.s.
(*E*)-β-farnesene	2.386	n.s.	3.419	0.046	0.815	n.s.
(*E,E*)-α-farnesene	1.913	n.s.	35.035	0.000	1.913	n.s.
β-selinene	1.316	n.s.	14.356	0.000	6.855	0.004
α-selinene	1.925	n.s.	16.654	0.000	7.681	0.002
β-bisabolene	1.441	n.s.	58.889	0.000	1.441	n.s.
γ-cadinene	9.074	0.005	21.609	0.000	9.074	0.001
δ-cadinene	2.506	n.s.	14.467	0.000	5.509	n.s.
total volatiles	4.363	0.045	5.707	0.008	1.463	n.s.

In 2003 the volatile blend included again β- and α-selinene but furthermore α-ylangene, (*E*)-α-bergamotene, and (*E*)-β-farnesene. The sesquiterpene β-bisabolene could only be detected in this year.

To examine differences between single years, post hoc tests were performed. By comparing the years 2001 and 2002, significant differences in emission were found for linalool, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, β-selinene, α-selinene, and γ- and δ-cadinene (table 2.16). This can be explained by the above illustrated qualitative change in the volatile composition (tables A 29 and A 30). However, the monoterpenoid alcohol linalool could be detected in both years but there was a strong decrease in emission in 2002 relative to the year before (tables A 29 and A 30). The comparison of 2001 and 2003 showed significant differences for the terpenes (*E*)-β-ocimene, (*E*)-β-caryophyllene, (*E,E*)-α-farnesene, β-selinene, α-selinene, β-bisabolene, and γ- and δ-cadinene (table 2.16). Even though (*E*)-β-ocimene was present in both years, the emission showed considerable quantitative differences such that in 2003 higher amounts of this monoterpene were released. The differences found for the sesquiterpenes can be explained by the qualitative change of the volatile profile among the years. The total amount emitted was significantly increased in 2003 compared to 2002

as in 2003 the volatile blend included a higher number of terpenes and also the amounts in emission of the individual terpenes were enhanced relative to 2002 (table 2.16). Moreover, the sesquiterpenes (*E*)-α-bergamotene and β-bisabolene showed significant differences between 2002 and 2003 since they were present in 2003 but not in 2002 (tables 2.16 and A 30).

Table 2.16. Effects of the years on the volatile emission of the corn cultivars Nobilis (isogenic) and Novelis (transgenic) in the field near Halle in 2001

Post hoc tests showed differences in mean volatile emission (ng/h/plant) and p-values. Negative diff. of means indicate higher amounts of the second group within each comparison, while positive diff. of means represent higher amounts of the first group. Empty fields: these compounds could not be detected in both years. n.s.: not significant (p>0.05). D: Dunnett's T3 test; T: Tukey; N = 6.

volatile compound	post hoc test	2001 vs 2002		2001 vs 2003		2002 vs 2003	
		diff. means	p	diff. means	p	diff. means	p
limonene	**T**	15.197	n.s.	-2.911	n.s.	-18.108	n.s.
(*E*)-β-ocimene	**T**	-129.706	n.s.	-157.873	0.035	-28.167	n.s.
linalool	**D**	349.858	0.014	136.753	n.s.	-213.106	n.s.
DMNT	**T**	-71.287	n.s.	-679.924	0.002	-608.924	n.s.
α-ylangene	**D**	3.007	n.s.	2.752	n.s.	-0.256	n.s.
(*E*)-β-caryophyllene	**D**	454.627	0.003	454.627	0.003		
(*E*)-α-bergamotene	**D**	221.129	0.020	-53.326	n.s.	-274.455	0.005
(*E*)-β-farnesene	**D**	352.762	0.002	-342.297	n.s.	-695.059	n.s.
(*E*,*E*)-α-farnesene	**D**	148.731	0.000	148.731	0.000		
β-selinene	**D**	-134.271	0.006	-65.848	0.001	68.422	n.s.
α-selinene	**D**	-168.060	0.004	-83.242	0.001	84.818	n.s.
β-bisabolene	**D**			-130.697	0.000	-130.697	0.000
γ-cadinene	**D**	13.735	0.014	13.735	0.014		
δ-cadinene	**D**	28.460	0.013	28.460	0.013		
total volatiles	**D**	1084.182	n.s.	-731.662	n.s.	-1815.243	0.006

Interactions between Bt-transformation and years. Both the transformation with Bt and the year played an important role and considerably influenced the volatile profile of the maize varieties in the field. As shown in table 2.15 and figure 2.12, interactions of both factors were significant for the homoterpene DMNT as well as for the sesquiterpenes α-ylangene, β- and α-selinene, and γ-cadinene. DMNT showed contrary trends in 2003 in comparison to the years before: while it was released in elevated amounts by the isogenic corn line relative to the transgenic corn line in 2001 and 2002, which was found to be significant only for 2002, the amount released by the transgenic cultivar dramatically increased in 2003 thus tending to result in a higher amount compared to the isogenic line (figure 2.12). Despite the complete lack of β- and α-selinene in 2001, these volatiles showed a comparable tendency for 2002 and 2003. Both sesquiterpenes were released in 2002 in significant higher amounts by the isogenic cultivar relative to the transgenic cultivar. Since the release by the isogenic corn line dramatically decreased from 2002 to 2003 and the transgenic cultivar showed similar emissions in both years, the isogenic corn line emitted significantly lower amounts of these compounds than the transgenic corn line in 2003 (figure 2.12). The sesquiterpene α-ylangene could not be detected in 2002 and showed qualitative differences between both cultivars as it was exclusively emitted by the isogenic line in 2001 and 2003. Gamma-cadinene, on the other hand, was only released in 2001 and showed a significant elevated emission by the isogenic cultivar relative to the transgenic cultivar (figure 2.12).

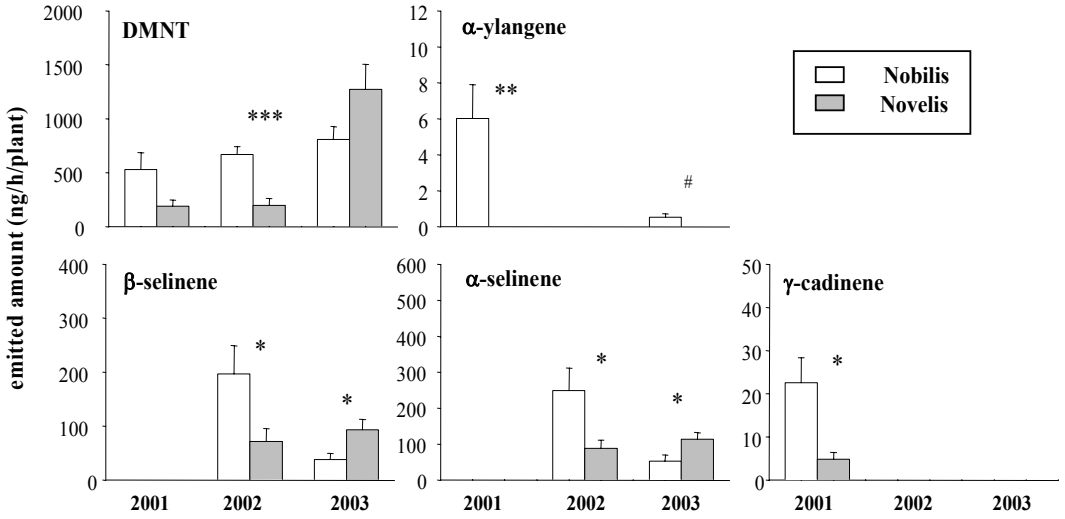

Figure 2.12. Emitted volatiles showing interactions between years and transformation with the Bt-toxin in the lines Nobilis (isogenic) and Novelis (transgenic) in August 2001 to 2003

Stars (t-test) and double crosses (U-test) indicate significant differences between the lines within the same treatment (*/ #: p<0,05; **/ ##: p<0,01; ***/ ###: p<0,001); N = 6.

Hence, seven out of fourteen compounds and the total volatiles were significantly influenced in their release by the transformation with a Bt-coding gene. Two volatiles - α-ylangene and γ-cadinene - as well as the total amount emitted showed differences between both cultivars among all years, whereas five terpenes - DMNT, α-ylangene, β-selinene, α-selinene, and γ-cadinene - were affected in certain years.

Proportions of the individual components within the volatile blend. There were highly significant differences in the volatile composition and therefore the proportions of the individual components between the cultivars within each month and between the months within each corn line (table 2.17). Interestingly, the differences in the composition of volatile blend between the corn lines within the each month was less pronounced than the differences between each month within the cultivars.

Table 2.17. Comparison of the volatile composition released by the corn cultivars Nobilis (isogenic) and Novelis (transgenic) in the field in August 2001 to 2003

Results of the χ^2-statistics are shown for the comparison of the volatile emission between the years within the pair of cultivar as well as for each cultivar. n.t.: not tested. The numbers above the diagonal line represent the p-values, and the numbers below the diagonal line represent the χ^2-values.

χ^2 \ p		2001		2002		2003	
		Nobilis	Novelis	Nobilis	Novelis	Nobilis	Novelis
2001	Nobilis		<0.001	<0.001	n.t.	<0.001	n.t.
	Novelis	10.07		n.t.	<0.001	n.t.	<0.001
2002	Nobilis	95.51	n.t.		<0.001	<0.001	n.t.
	Novelis	n.t.	69.76	12.85		n.t.	<0.001
2003	Nobilis	44.32	n.t.	69.83	n.t.		<0.001
	Novelis	n.t.	60.72	n.t.	44.22	16.71	

2.3.2.2. Comparison between Prelude (isogenic) and Valmont (transgenic)

Since there were differences between the lines Nobilis and Novelis, another pair of cultivar - Prelude and Valmont - was studied throughout the field seasons 2002 and 2003

in the field near Halle (see table 2.4). In these years, the volatile profile included β-myrcene, (*Z*)-3-hexen-1-yl acetate, limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, β-selinene, α-selinene, α-farnesene, γ-cadinene, and δ-cadinene and no qualitative differences between both lines could be found (table 2.18). The average emission and standard deviations of all individual compounds and the total volatiles are shown in tables A 31 and A 32 in the appendix. The release of these compounds was examined with regard to a possible influence of the factors Bt-transformation, months and years.

Table 2.18. Effects of years and months on the volatile emission of the corn lines Prelude (isogenic) and Valmont (transgenic) in the field near Halle
F-values and p-values are shown for differences between months, years, and interactions. month: June to August; year: 2002 and 2003; n.s.: not siginificant (p>0.05); N = 6.

volatile compound	month (dF=2)		year (dF=1)		interaction (year x month)	
	F	p	F	p	F	p
β-myrcene	11.632	0.000	5.766	0.019	8.952	0.000
(*Z*)-3-hexen-1-yl acetate	57.689	0.000	42.685	0.000	42.685	0.000
limonene	6.089	0.004	2.722	n.s.	9.025	0.000
(*E*)-β-ocimene	43.599	0.000	0.403	n.s.	5.431	0.007
linalool	10.923	0.000	21.9	0.000	7.611	0.001
DMNT	9.091	0.000	38.349	0.000	4.347	0.017
α-ylangene	20.569	0.000	82.763	0.000	13.528	0.000
cycloisosativene	27.264	0.000	27.264	0.000	27.264	0.000
(*E*)-β-caryophyllene	10.914	0.000	1.544	n.s.	10.012	0.000
(*E*)-α-bergamotene	19.790	0.000	0.877	n.s.	0.877	0.000
(*E*)-β-farnesene	15.742	0.000	14.266	0.000	4.261	0.019
(*E,E*)-α-farnesene	13.988	0.000	1.782	n.s.	1.782	n.s.
β-selinene	22.670	0.000	0.138	n.s.	2.413	n.s.
α-selinene	13.692	0.000	1.123	n.s.	1.215	n.s.
γ-cadinene	19.660	0.000	15.881	0.000	7.054	0.002
δ-cadinene	12.642	0.000	6.748	0.012	4.095	0.022
total volatiles	39.911	0.000	45.075	0.000	11.632	0.000

Factor month. As shown in table 2.18, significant differences between the months were found for all individual volatiles as well as for the total emission independently of the years and the transformation with a Bt-coding gene. By comparing the volatile emission in June and July, the release of β-myrcene, (*Z*)-3-hexen-1-yl acetate, (*E*)-β-ocimene, cycloisosativene, (*E*)-α-bergamotene, (*E*)-α-farnesene, and β- and α-selinene showed significant differences (table 2.19). Beta-myrcene and β-ocimene were emitted by the plants in lower amounts in June compared to July while the compounds (*E*)-α-bergamotene, β- selinene, and α-selinene could not be detected in June but were emitted in July. (*Z*)-3-hexen-1-yl acetate, cycloisosativene and (*E*)-α-farnesene, on the other hand, were exclusively released in June (tables A 31 and A 32). The comparison between June and August showed significant differences in emission for the total amount and all individual volatiles except for β-myrcene, limonene and (*E*)-α-bergamotene (table 2.19). The total volatiles emitted and the volatiles (*Z*)-3-hexen-1-yl acetate, linalool, DMNT, α-ylangene, cycloisosativene, (*E*)-β-caryophyllene, (*E*)-β- and (*E,E*)-α-farnesene, γ- and δ-cadinene showed higher amounts in June than in August, while (*E*)-β-ocimene and β- and α-selinene were emitted in lower amounts in June compared to August (tables A 31 and 2.19). In July, the total volatiles as well as the terpenes limonene, (*E*)-β-ocimene, linalool,

DMNT, α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, and γ- and δ-cadinene showed significantly higher amounts than in August (table 2.19).

Table 2.19. Effects of the different months on the volatile emission of the corn cultivars Prelude (isogenic) and Valmont (transgenic) in the field near Halle
Post hoc test (Dunnet's T3) showed differences in mean volatile emission (ng/h/plant) and p-values. Negative diff. of means indicate higher amounts of the second group within each comparison, while positive diff. of means represent higher amounts of the first group. n.s.: not significant (p>0.05); N = 6.

volatile compound	June vs July		June vs August		July vs August	
	diff. means	p	diff. means	p	diff. means	p
β-myrcene	-32.964	0.046	-1.165	n.s.	31.799	n.s.
(*Z*)-3-hexen-1-yl acetate	975.316	0.000	975.316	0.000	0.000	n.s.
limonene	-93.199	n.s.	40.469	n.s.	133.669	0.011
(*E*)-β-ocimene	-343.629	0.000	-49.478	0.020	294.151	0.000
linalool	-100.025	n.s.	253.047	0.043	353.072	0.000
DMNT	-9.131	n.s.	274.884	0.041	284.014	0.001
α-ylangene	3.225	n.s.	6.758	0.001	3.534	0.038
cycloisosativene	92.712	0.004	92.712	0.004	0.000	n.s.
(*E*)-β-caryophyllene	-314.385	n.s.	143.705	0.013	458.090	0.010
(*E*)-α-bergamotene	-275.080	0.001	0.000	n.s.	275.080	0.001
(*E*)-β-farnesene	17.023	n.s.	267.665	0.001	250.642	0.000
(*E,E*)-α-farnesene	151.444	0.003	151.444	0.003	0.000	n.s.
β-selinene	-50.596	0.000	-72.874	0.000	-22.278	n.s.
α-selinene	-102.689	0.000	-68.453	0.000	34.237	n.s.
γ-cadinene	5.398	n.s.	18.791	0.000	13.393	0.000
δ-cadinene	13.418	n.s.	33.686	0.002	20.680	0.000
total volatiles	-49.406	n.s.	2073.879	0.000	2123.284	0.000

Factor year. After combining the data for different months and Bt-transformation significant differences between the years were found for a variety of compounds (table 2.18): the volatiles (*Z*)-3-hexen-1-yl acetate, linalool, DMNT, α-ylangene, (*E*)-β-farnesene, γ- and δ-cadinene, and the total volatiles showed higher emitted amounts in 2003 compared to 2002 (tables A 31 and A 32). Beta-myrcene, however, was released in 2003 in lower amounts than in 2002 and cycloisosativene was only detected in June 2002.

Interactions between year and month. Regardless of the Bt-transformation, significant interactions between years and months were found for the total amount emitted as well as for most volatiles except for (*E,E*)-α-farnesene, β-selinene, and α-selinene (table 2.18). Whereas the total emission continuously decreased over the field season in 2003, in the year 2002 another tendency was found. Here, the total amount increased from June to July and drastically decreased in August by the factor seven (tables A 31 and A 32). Also, there were quantitative as well as qualitative changes in the volatile profiles of the plants among the months and the years (tables 2.18 and A 31 and A 32; figure 2.13). The green leaf volatile (*Z*)-3-hexen-1-yl acetate, for instance, was released only in June of both years, whereas (*E*)-α-bergamotene could only be detected in July of both years (tables A 31 and A 32). Otherwise, limonene, DMNT, and α-ylangene were emitted in both years over the whole field season. Other volatile compounds such as β-myrcene, linalool, (*E*)-β-caryophyllene, (*E*)-β-farnesene, β-selinene, or α–selinene showed different emission patterns between the years (tables A 31 and A 32).
These results demonstrate that the volatile profile changed considerably and no consistent pattern regarding the up- or down regulation of individual volatile compounds was found among each field season as well as among the years.

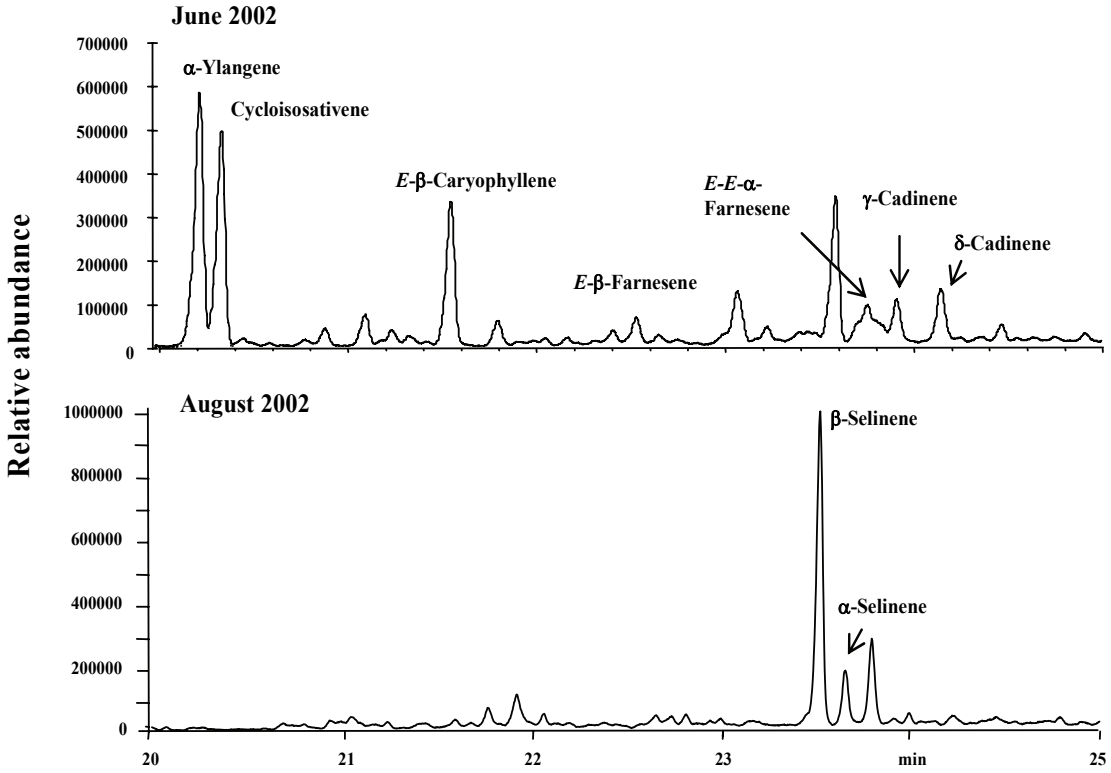

Figure 2.13. Sesquiterpene volatiles of the cultivar Prelude showing differences in the profile between the months June 2002 and August 2002 in the field near Halle

Factor Bt-transformation. After combining the data of years and months no significant differences between the transgenic line and the isogenic line could be detected (table 2.20).

Interactions between Bt-transformation, months and years. After combining the data of the different months, significant interactions between transformation with Bt and year were found for the monoterpene β-myrcene (table 2.20) which was emitted by both lines in equal amounts in 2003. In 2002, this compound showed a 10-fold increase in the isogenic line over that emitted by the transgenic line (table A 31).
Independently of the year, significant interactions between Bt and month were shown for both β-myrcene and the sesquiterpene β-selinene. While β-myrcene was emitted by both corn lines in equal amounts in June and August, a strong increase in emission by the isogenic line was found relative to the transgenic line in July. The sesquiterpene β–selinene, on the other hand, was not detected in June but in July and August. Whereas it was emitted in equal amounts in July by both lines the emission in August was strongly increased in the transgenic line compared to that emitted by the isogenic line (tables A 31 and A 32). Finally, significant interactions between the factors Bt, year and month were found for β-myrcene and (*E*)-β-caryophyllene (table 2.20). This can be explained by the above illustrated differences in emission between both cultivars and among the months and years (see also tables A 31 and A 32).

Consequently, three of sixteen compounds were significantly affected in their release by the transformation with a Bt-coding gene although this influence only was found in combination with the factors year and/ or month. Beta-selinene showed differences between both cultivars in certain months, and another terpene - β-myrcene - was affected

in certain years and months, whereas (*E*)-β-caryophyllene as well as β-myrcene were influenced by all factors (table 2.20).

Table 2.20. Results of the Three-factorial Analysis of Variance for the effects of transformation with Bt, first-order interactions with the factors year and month, and second-order interactions on the volatile emission in the corn lines Valmont (transgenic) and Prelude (isogenic) in the field near Halle

F-values and p-values are shown for differences between the isogenic line Prelude and the transgenic line Valmont, years, months, and interactions. year: 2002 and 2003. month: June to August. n.s.: not significant ($p>0.05$); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		interaction (Bt x year)		interaction (Bt x month)		interaction (Bt x year x month)	
	F	p	F	p	F	p	F	p
β-myrcene	3.236	n.s.	6.797	0.012	6.450	0.003	6.837	0.002
(*Z*)-3-hexen-1-yl acetate	0.08	n.s.	0.200	n.s.	0.347	n.s.	0.200	n.s.
limonene	0.624	n.s.	0.417	n.s.	0.224	n.s.	0.014	n.s.
(*E*)-β-ocimene	0.194	n.s.	0.014	n.s.	0.699	n.s.	0.089	n.s.
linalool	0.083	n.s.	1.348	n.s.	0.307	n.s.	0.287	n.s.
DMNT	0.848	n.s.	30.41	n.s.	1.170	n.s.	0.652	n.s.
α-ylangene	0.093	n.s.	0.844	n.s.	0.541	n.s.	0.994	n.s.
cycloisosativene	0.110	n.s.	0.310	n.s.	0.310	n.s.	0.310	n.s.
(*E*)-β-caryophyllene	1.885	n.s.	2.885	n.s.	2.536	n.s.	3.611	0.033
(*E*)-α-bergamotene	0.415	n.s.	1.685	n.s.	0.643	n.s.	1.683	n.s.
(*E*)-β-farnesene	0.873	n.s.	0.999	n.s.	0.401	n.s.	0.394	n.s.
(*E,E*)-α-farnesene	0.432	n.s.	0.077	n.s.	0.584	n.s.	0.077	n.s.
β-selinene	2.749	n.s.	0.234	n.s.	3.772	0.029	0.058	n.s.
α-selinene	1.539	n.s.	0.309	n.s.	0.620	n.s.	0.274	n.s.
γ-cadinene	0.423	n.s.	0.004	n.s.	0.750	n.s.	0.683	n.s.
δ-cadinene	0.391	n.s.	1.397	n.s.	1.673	n.s.	0.412	n.s.
total volatiles	0.000	n.s.	0.161	n.s.	0.777	n.s.	0.446	n.s.

Proportions of the individual components within the volatile blend. As illustrated in table 2.21 and figure 2.14, there were highly significant differences in the proportions of the individual components between years, between months within each cultivar as well as between the cultivars. Remarkably, the differences in volatile composition between transgenic and isogenic corn line within months and years are by far smaller than the differences between years and between months within each cultivar. The greatest differences were found for both corn lines between the months in 2002 (table 2.21). As demonstrated above, the volatile profile of the cultivars changed qualitatively within the field season 2002: while in June and July the volatile blend included a variety of components, most of these volatiles were not detected in August thus leading to a strong decrease in the total emission (figures 2.13 and 2.14). Looking at the different classes of volatiles, this led to a change of the percentage of the monoterpene-fraction and sesquiterpene-fraction within the scent of both cultivars. In June, the profile consisted of up to 20 % green leaf volatiles, mono- and homoterpenes and of about 80 % sesquiterpenes, whereas in July the percentage of the green leaf volatiles, mono- and homoterpenes increased to almost 75 % (figure 2.14). In August of the same year, only four compounds were present and approximately 50 % of the volatile profile consisted of mono- and homoterpenes, and the other half being sesquiterpenes. A different pattern appeared in 2003. Whereas in June the volatile profile consisted of more than 75 % green leaf volatiles, mono- and homoterpenes, in July their amount dropped to 40 % in the

isogenic line and 55 % in the transgenic line to increase again in August to about 80 % (figure 2.14).
Otherwise, less variations between transgenic and isogenic corn lines were found in all months of both years. Two exceptions were found: (*E*)-β-caryophyllene was released in July 2003 by the isogenic corn line Prelude with much higher percentage of total than by the transgenic line, while the opposite trend was found for β-selinene in August 2002. As a result, the months and years influenced the volatile composition by far more than the cultivar.

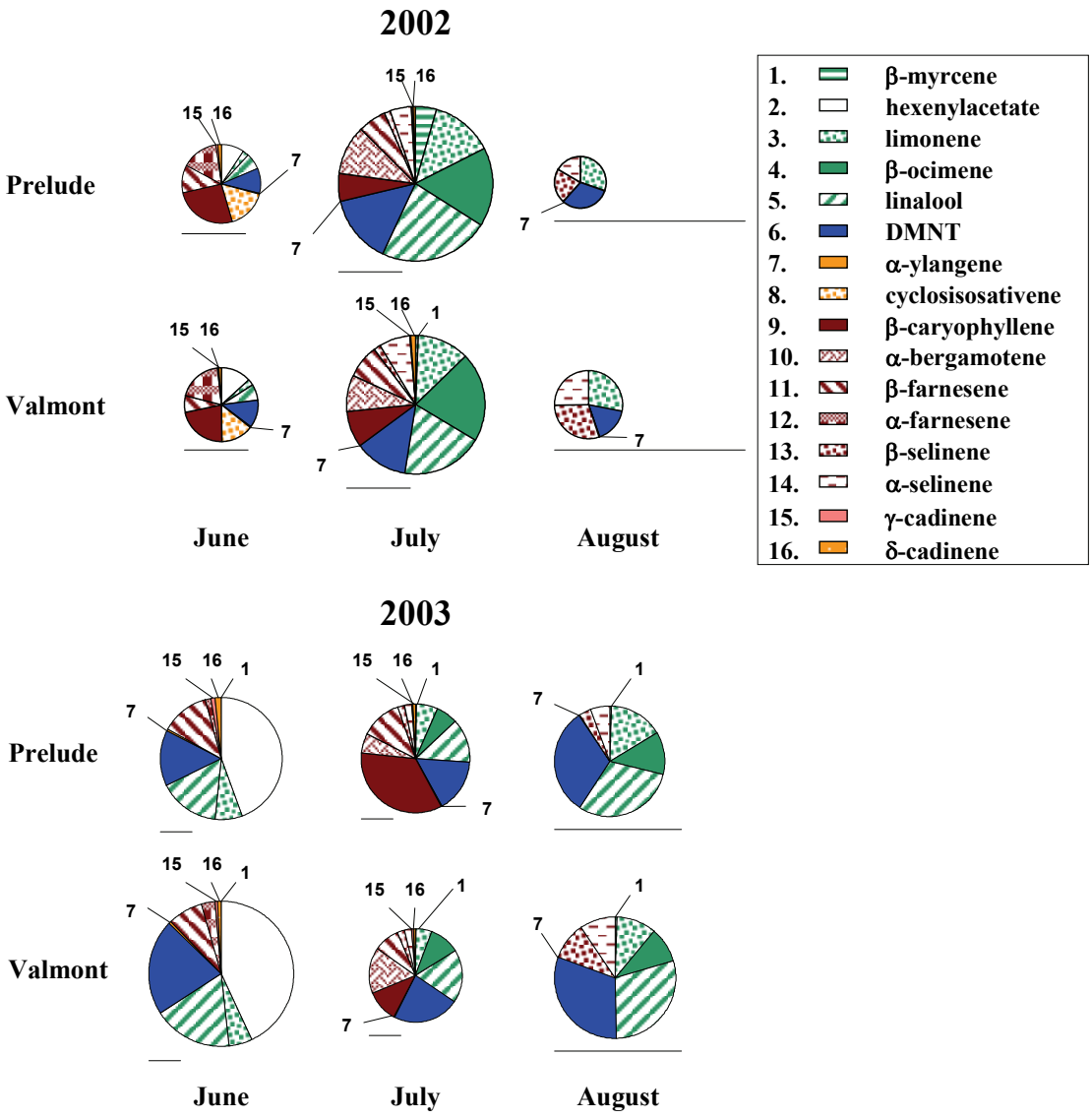

Figure 2.14. Comparison of the volatile emission by the corn cultivars Prelude (isogenic) and Valmont (transgenic) in the field 2002 and 2003
The mean percentages are shown for the individual volatiles. White represents the green leaf volatiles, green colours the monoterpenes, blue colours the homoterpenes, red colours the sesquiterpenes induced by herbivory, and orange colours the remaining sesquiterpenes. The line beneath each circle corresponds to an amount of 1 µg and the diameter of each circle corresponds to the total amount emitted (ng/h/plant). Numbers indicate those volatiles released below 1.5% of the total emission.

Table 2.21. Comparison of the volatile composition released by the corn cultivars Prelude (isogenic) and Valmont (transgenic) in the field in 2002 and 2003

Results of the χ^2-statistics are shown for the comparison of the volatile emission between months and years within the pair of cultivar as well as for each cultivar. n.t.: not tested. The numbers above the diagonal line represent the p-values, and the numbers below the diagonal line represent the χ^2-values.

χ^2 / p			2002						2003					
			Prelude			Valmont			Prelude			Valmont		
			June	July	August	June	July	August	June	July	August	June	July	August
2002	Prelude	June		<0.001	<0.001	<0.001	n.t.	n.t.	<0.001	n.t.	n.t.	n.t.	n.t.	n.t.
		July	109.80		<0.001	n.t.	<0.001	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		August	159.40	104.60		n.t.	n.t.	<0.001	n.t.	n.t.	<0.001	n.t.	n.t.	n.t.
	Valmont	June	2.87	n.t.	n.t.		<0.001	<0.001	n.t.	n.t.	n.t.	<0.001	n.t.	n.t.
		July	n.t.	6.16	n.t.	102.97		<0.001	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
		August	n.t.	n.t.	7.61	161.30	108.63		n.t.	n.t.	n.t.	n.t.	n.t.	<0.001
2003	Prelude	June	83.33	n.t.	n.t.	n.t.	n.t.	n.t.		<0.001	n.t.	<0.001	n.t.	n.t.
		July	n.t.	37.98	n.t.	n.t.	n.t.	n.t.	97.29		<0.001	n.t.	<0.001	n.t.
		August	n.t.	n.t.	66.08	n.t.	n.t.	n.t.	96.47	73.82		n.t.	n.t.	<0.001
	Valmont	June	n.t.	n.t.	n.t.	71.55	n.t.	n.t.	3.83	n.t.	n.t.		<0.001	<0.001
		July	n.t.	n.t.	n.t.	n.t.	14.63	n.t.	n.t.	20.68	n.t.	88.69		<0.001
		August	n.t.	n.t.	n.t.	n.t.	n.t.	68.10	n.t.	n.t.	5.38	90.62	51.23	

2.3.2.3. Comparison between Antares (isogenic) and Navares (transgenic)

As illustrated above, the volatile profiles of all corn lines measured in the field near Halle changed qualitatively and quantitatively depending on year, month and transformation with Bt. To elucidate the question whether these changes could also be found for other cultivars, the corn lines Antares and Navares cultivated in the field site near Kitzingen were additionally examined. Furthermore it was of special interest whether the release of β- and α-selinene at the end of the field season 2002 and 2003 in Halle was a local phenomenon or also could be found in other regions.

- **The volatile blend within the field season 2003**

The volatile release of the transgenic corn line Navares and the isogenic corn line Antares were examined in July and August in the year 2003 in the field near Kitzingen (see table 2.4). The profile included β-myrcene, (*Z*)-3-hexen-1-yl acetate, limonene, linalool, DMNT, α-ylangene, (*E*)-β-caryophyllene, β- and α-selinene, and γ- and δ-cadinene (table 2.22). The release of these compounds was compared with regard to a possible influence of the factors Bt-transformation and months. The average release and standard deviations of all individual volatiles as well as of the total amount emitted are shown in table A 33 in the appendix.

Factor Bt-transformation. Irrespective of the months, significant differences between the transgenic and isogenic line were found for DMNT and δ-cadinene (table 2.22). Both compounds showed a contrary emission pattern: while DMNT was released in higher amounts by the transgenic corn line relative to the isogenic corn line, the release of δ-cadinene was increased in the isogenic cultivar (table A 33).

Factor month. By comparing the volatile emission between the months independently of the Bt-transformation, there were significant differences found for all volatiles except for the total amount and the terpene alcohol linalool (table 2.22). The sequiterpenes (*E*)-β-caryophyllene and γ- and δ-cadinene were exclusively emitted in July but not in August whereas the opposite was found for (*Z*)-3-hexen-1-yl acetate, (*E*)-β-ocimene, and β- and α-selinene (table A 33). Although β-myrcene, limonene and α-ylangene were released in both months, the emission decreased dramatically from July to August (table A 33). For DMNT, on the other hand, a 3-fold increase from July to August was observed.

Interactions between Bt-transformation and month. Both factors together, the Bt-transformation and the months, considerably affected the release of δ-cadinene as the only volatile influenced by this interaction (table 2.22). While this compound was released by neither cultivar in August, the isogenic line emitted in July almost twice the amount of δ-cadinene compared to the transgenic line (table A 33).

Given these results, only DMNT and δ-cadinene were significantly influenced by the Bt-transformation, whereas the release of most compounds was affected by the months (table 2.22). Moreover, the two sesquiterpenes β- and α-selinene found to be released by the cultivars Nobilis/Novelis and Prelude/Valmont in the late field season 2002 and 2003, were also detected (table A 33).

Table 2.22. Results of the Two-factorial Analysis of Variance for the effects of transformation with Bt, months, and first-order interactions on the volatile emission in the corn lines Navares (transgenic) and Antares (isogenic) in the field near Kitzingen
F-values and p-values are shown for differences between the isogenic line Antares and the transgenic line Navares, months, and interactions. month: July and August. n.s.: not significant ($p>0.05$); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		month (dF=1)		interaction (Bt x month)	
	F	p	F	p	F	p
β-myrcene	0.285	n.s.	23.846	0.000	1.904	n.s.
(*Z*)-3-hexen-1-yl acetate	2.823	n.s.	23.923	0.000	2.823	n.s.
limonene	0.281	n.s.	25.583	0.000	0.407	n.s.
(*E*)-β-ocimene	3.435	n.s.	223.712	0.000	3.435	n.s.
linalool	0.639	n.s.	0.853	n.s.	0.010	n.s.
DMNT	4.515	0.015	39.025	0.000	0.342	n.s.
α-ylangene	0.003	n.s.	63.500	0.000	0.003	n.s.
(*E*)-β-caryophyllene	0.613	n.s.	19.100	0.000	0.613	n.s.
β-selinene	2.824	n.s.	27.049	0.000	2.824	n.s.
α-selinene	2.500	n.s.	30.356	0.000	2.500	n.s.
γ-cadinene	1.834	n.s.	23.603	0.000	1.834	n.s.
δ-cadinene	21.505	0.000	27.300	0.000	21.505	0.000
total volatiles	1.188	n.s.	0.643	n.s.	1.159	n.s.

Proportions of the individual components within the volatile blend. The proportions of the components in the volatile mixtures showed highly significant differences for the comparison between Navares and Antares within each month but also between the months within each cultivar ($p<0.001$). Remarkably the volatile composition and the proportions of their components differed more between the months within transgenic and isogenic cultivar (χ^2-values 75.18 and 79.91, respectively) than between the lines within July or August (χ^2-values 1.88 and 2.26, respectively).

- **The volatile blend in the month August in the years 2002 and 2003**

The volatiles of the transgenic corn line Navares and the isogenic corn line Antares were analyzed at the end of the field seasons in 2002 and 2003 in the field near Kitzingen (see table 2.4). The volatile profile included β-myrcene, (*Z*)-3-hexen-1-yl acetate, limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, (*E*)-β-farnesene, and β- and α-selinene (table 2.23). The release of these compounds was examined with regard to a possible influence of the factors Bt-transformation and years. The average emission and standard deviations of all individual volatiles as well as of the total amount are shown in table A 33 in the appendix.

Factor Bt-transformation. After combining the data of the years, no significant differences between transgenic and isogenic varieties were found (table 2.23).

Factor year. Independently of the transformation with Bt, significant differences between the years were shown for β-myrcene, (*Z*)-3-hexen-1-yl acetate, limonene, (*E*)-β-ocimene, DMNT, α-ylangene, β-selinene and the total volatile amount (table 2.23). Beta-myrcene, limonene, α-ylangene and β-selinene were found to be decreased in 2003 compared to 2002 with the most pronounced effect for α-ylangene, which was released by only 15 % of the amount detected in 2002 (table 2.23 and A 33). On the other hand, β-ocimene, DMNT and the total volatiles were emitted up to 4-fold higher in 2003 compared to 2002 (table A 33). In addition to the above demonstrated quantitative differences between the years, there were also qualitative differences such that β-

farnesene was only released in 2002, whereas (*Z*)-3-hexen-1-yl acetate could only be found in 2003.

Interactions between Bt-transformation and year. Although there were some variations in the release of several individual compounds, no significant interactions between transformation with Bt and the year were found (table 2.23).

These results show that significant influences on the volatile release were found between the years, whereas the transformation with Bt had no effect. Furthermore, the two sesquiterpenoid compounds β-selinene and α-selinene also could be detected in the cultivars Navares/Antares at the end of each field season.

Table 2.23. Results of the Two-factorial Analysis of Variance for the effects of transformation with Bt, years, and first-order interactions on the volatile emission in the corn lines Navares (transgenic) and Antares (isogenic) in the field near Kitzingen
F-values and p-values are shown for differences between the isogenic line Antares and the transgenic line Navares, years, and interactions. year: 2002 and 2003. n.s.: not significant (p>0.05); N = 6.

volatile compound	Bt vs non-Bt (dF=1)		year (dF=1)		interaction (Bt x year)	
	F	p	F	p	F	p
β-myrcene	0.175	n.s.	46.585	0.000	0.800	n.s.
(*Z*)-3-hexen-1-yl acetate	2.823	n.s.	23.923	0.000	2.823	n.s.
limonene	1.308	n.s.	20.111	0.000	1.714	n.s.
(*E*)-β-ocimene	1.163	n.s.	92.602	0.000	4.257	n.s.
linalool	0.510	n.s.	0.330	n.s.	0.001	n.s.
DMNT	2.237	n.s.	51.293	0.000	1.794	n.s.
α-ylangene	1.024	n.s.	22.055	0.000	2.897	n.s.
(*E*)-β-farnesene	0.978	n.s.	2.911	n.s.	0.978	n.s.
β-selinene	3.684	n.s.	4.676	0.046	0.678	n.s.
α-selinene	2.535	n.s.	1.049	n.s.	0.333	n.s.
total volatiles	3.182	n.s.	12.041	0.002	0.917	n.s.

Proportions of the individual components within the volatile blend. The volatile composition showed highly significant differences between the corn lines within one year as well as between the years within the corn lines ($p<0.001$). There were greater differences between the years within the isogenic and transgenic cultivar (χ^2-values 57.38 and 39.80, respectively) than between transgenic and isogenic cultivar within 2002 or 2003 (χ^2-values 1.76 and 2.26, respectively).

2.3.3. Parasitation rates in the field

To examine possible effects of the introduced Bt-coding gene on the tritrophic interaction, cages with larvae of *Spodoptera littoralis* feeding on plant material of the corresponding maize line were distributed among all corn lines in both field sites and removed after one week to the laboratory. To visualize possible parasitations, the larvae were reared on artificial diet until pupation. In both fields, no parasitized larvae of *S. littoralis* were found. However, in 48 % of the cages perished parasitoids were found whose distribution showed no significant differences between the transgenic corn lines and their corresponding isogenic lines (t-test; $p>0.05$).

2.4. Discussion

This study was aimed to elucidate the question whether the transformation of corn lines with a Bt-coding gene might change the volatile profile of the plants, which affects the tritrophic system consisting of maize plants, folivorous lepidopteran larvae and parasitic wasps. Since the target-lepidopteran *Ostrinia nubilalis* is rapidly killed by the endogenous Bt-toxin, non-target pest insects such as the turnip moth *Agrotis segetum* might feed for a longer time on the maize plants and could induce another volatile pattern due to different feeding habits or the presence of a special elicitor in their regurgitant. To answer these open questions, several transgenic corn lines derived from two different Bt-events and their corresponding isogenic lines were infested in laboratory experiments with different herbivores and the volatile profiles emitted by these plants were analyzed. The cultivars Novelis (MON810)/ Nobilis were exposed to feeding by *S. littoralis*, Navares (Bt176)/ Antares experienced damage by *S. littoralis* or *O. nubilalis*, and the varieties Valmont (Bt176)/ Prelude were infested by the herbivores *S. littoralis*, *O. nubilalis* or *A. segetum*.
The volatile profile of the two Bt176-lines (Valmont and Navares) differed in neither treatment qualitatively from those emitted by their corresponding isogenic lines but significant quantitative differences were found. These differences varied considerably depending on the particular treatment. For example, after infestation of the cultivars Valmont and Prelude with *A. segetum*, a clear trend towards a higher total emission in the transgenic corn line than in the isogenic line was observed. Consequently, it is very surprising that the larvae of this herbivore, which should be barely susceptible to the Bt-toxin, fed less on the transgenic plants than on the isogenic plants (personal observation). This is supported by a study by Saxena and Stotzky (2001) who demonstrated on plants of the event Bt176 and their isogenic line that the introduced Bt-coding gene led to a significant higher lignin-content in the vascular bundle sheaths and in the sclerenchyma cells surrounding the vascular bundle, thus probably leading to plant material which is harder to chew for herbivores feeding on the stem such as *A. segetum*. However, less feeding damage but elevated volatile emission led to the assumption that the volatile release of the transgenic line might be more inducible by infestation with *A. segetum* than that of the isogenic corn line. A similar picture was shown for the comparison of these maize lines after infestation by larvae of the European corn borer *O. nubilalis*. Here, the same or in some cases higher amounts of most volatiles were released by the transgenic cultivar in comparison to the corresponding isogenic line even though the *Ostrinia*-larvae were rapidly killed by the endogenous Bt-toxin and barely destroyed plant tissue (personal observation). However, the results show that the extend of damage by the European cornborer seem to hardly influence the volatile emission of the maize plants (see also Turlings et al., 1998b) as discussed already for *Agrotis*-feeding, or the introduction of the Bt-gene into this cultivar may positively affect its ability to produce volatiles. This seems to be no general pattern since Navares, the other Bt176-line, showed the opposite tendency: after infestation by *O. nubilalis* the majority of the volatile compounds were emitted in lower amounts compared to the isogenic line. After exposure of both Bt-176-plants (Valmont/ Navares) and their corresponding controls (Prelude/ Antares) to *S. littoralis*, the transgenic corn lines emitted higher amounts of total volatiles and most individual compounds relative to the corresponding isogenic lines. The opposite trend was found for (*E*)-β-caryophyllene, but only for the pair of cultivars Prelude/Valmont, where the isogenic line released significant elevated amounts. This indicates a highly specific reaction to herbivory in the transgenic lines even within the same Bt-event.
On the other hand, the transgenic line Novelis derived from the Bt-event MON810 and the corresponding isogenic line Nobilis differed quantitatively as well as qualitatively in

the volatile blend emitted by *Spodoptera*-infested or control plants. Most remarkably, the sesquiterpenes α-ylangene and β-sesquiphellandrene could only be detected in the isogenic cultivar. Furthermore, after infestation by *S. littoralis* the transgenic line showed an inconsistent induction of the individual volatiles: six of 13 volatiles including the typically herbivory-induced terpenes (*E*)-β-caryophyllene and (*E*)-β-farnesene as well as the total amount were released in lower amounts compared to the isogenic line. In contrast, the terpene alcohol linalool was strongly increased in the transgenic line relative to the isogenic line, both in the control plants and after herbivory. These differences in emission between the isogenic and transgenic corn lines can not be explained by a reduced damage by *S. littoralis* as they fed as readily on the Bt-corn as on the non-transgenic cultivar (personal observation). Hence, the introduction of the event MON810 into the maize plants considerably impaired the composition of the volatile blend as well as the quantity of emission. This reduction in total emission could be the result of a different resource allocation in the transgenic line by which resources usually used for the synthesis of primary or secondary metabolites may be needed for the production of the Bt-toxin. This is supported by another study on MON810, also part of this safety research. Büchs and his group (BBA, Braunschweig; Germany) examined the development of fungus gnats feeding on plant material from MON810-maize plants and found a delay in development and pupation even though the insects fed more of the transgenic plant material (public available data on http://www.biosicherheit.de/mais/308.doku.html). In contrast, fungus gnats reared on plant material of Bt176-corn did not show such an impact on the development. As a result, the effect found for MON810 cannot directly be attributed to the Bt-toxin itself but rather to the food quality of the MON810-plants. Recently, also Turlings et al. (2005) reported on the transgenic corn line Bt11 that expresses similar to MON810 the Cry1Ab-gene a strong decrease in emission compared to the isogenic line. Although the authors induced the plants by mechanical wounding and application of regurgitant to both corn lines, and not by infestation with herbivores, the trend is similar for MON810 and Bt1: both transgenic lines seem to be impaired in their synthesis of secondary metabolites such as terpenes after the introduction of a Bt-coding gene. Resulting from the very variable volatile emission of all three transgenic cultivars in comparison to the corresponding isogenic line after damage by the lepidopteran herbivores *S. littoralis*, *A.segetum* and *O. nubilalis*, the volatile release of the MON810- and Bt176-plants after feeding by other lepidopteran pest insects needs to be examined in more detail.

Remarkably, the infestation with various herbivorous insects appeared to cause greater differences in both the volatile profile and the emission level of the transgenic plants and their isogenic lines than the introduction of the Bt-coding gene itself, as almost all volatiles showed significant differences between treatments. As reported by De Moraes et al. (1998) on maize plants, there were qualitatively different volatile mixtures after feeding of the two closely related herbivores *Heliothis virescens* and *Helicoverpa zea*. Contrary, Turlings et al. (1998b) reported only on quantitative differences in the volatile blend of maize seedlings damaged by the folivorous caterpillar *S. littoralis* or the stem borer *O. nubilalis*. In their study, the plants released the same volatile blend but corn plants infested by *S. littoralis* emitted much higher amounts than those exposed to feeding by *O. nubilalis*. The present study partially contradicts the investigations by Turlings et al. (1998b), since beside quantitative also qualitative differences in the composition of the volatile blend were found after infestation of corn plants with the herbivores *O. nubilalis*, *A.segetum* and *S. littoralis*. For example, after *Spodoptera*-infestation, the cultivars Prelude and Valmont released among other volatiles an additional volatile compound, the sesquiterpene β-bisabolene, which could not be detected in the maize plants damaged by

O. nubilalis or *A. segetum*, or in uninfested control plants. On the other hand, plants of the same corn lines exposed to *Agrotis*-feeding emitted the sesquiterpene cycloisosativene, which was found neither after *Spodoptera*-feeding nor after *Ostrinia*-damage. Furthermore, in contrast to the plants damaged by *S. littoralis* or *O. nubilalis*, these plants released no δ-cadinene. Other qualitative and quantitative differences were found for the pair of cultivar Antares/ Navares. These cultivars released after damage by the lepidopteran herbivores *S. littoralis* or *O. nubilalis* some additional terpenes in comparison to the control plants while other volatiles were exclusively released after *Spodoptera*-feeding. Several studies have shown that the oral secretions of lepidopteran herbivores contain elicitors, which induce the synthesis and emission of volatile compounds very specifically (Mattiacci et al., 1995; Alborn et al., 1997; Pohnert et al. 1999). Hence, the qualitative differences in the volatile composition between plants exposed to *S. littoralis*, *O. nubilalis* or *A. segetum* might be the consequence of the presence of different elicitors in their regurgitant. Despite the variation in the volatile composition after damage by the different herbivores, there were large quantitative differences found in the pair of cultivar Prelude/ Valmont, showing that plants exposed to *S. littoralis* released the highest amounts and plants after *Agrotis*- or *Ostrinia*-feeding released comparable amounts at low levels. This might be explained by the feeding habits of these larvae (see also Turlings et al., 1998b): while the folivorous insect *S. littoralis* extensively destroys the plant material by feeding mainly on the leaves, stemborers such as *O. nubilalis* primarily drill into the plant and only damage at the point of entering. Although the turnip moth *A. segetum* is also feeding on leaves, in the present study it rather damaged the corn stalk curtly above the ground by feeding holes into it (personal observation). As a result, the comparable amounts emitted both by *Ostrinia*- and *Agrotis*-infested plants might be explained by their related feeding habit. All these qualitative and quantitative differences led to highly significant differences in the proportions of the individual volatile within the blend between the cultivars as well as within each cultivar.
Even though in BT176 and MON810, there were significant quantitative and in MON810 also qualitative differences found compared to their corresponding controls, the overall volatile emission pattern by the transgenic maize lines and their isogenic lines can be considered normal laying within the bounds of conventional maize lines (see also Köllner et al., 2004 and Gouinguene et al., 2001). However, the question arises, which consequences these variations could have regarding the function of these volatile blends such as influence on tritrophic systems. It was shown by Hoballah et al. (2002) that not only the quantitity of emitted volatiles is important for the attractiveness of herbivore-damaged plants for parasitoids but also the composition of the volatile blend, especially of the terpenoid fraction. Turlings et al. (2005), however, found no differences in the attraction of herbivore-infested transgenic and isogenic corn plants to parasitoids although the Bt-corn showed a strong reduction in the volatile release relative to the isogenic line. As illustrated in the present study, there were highly significant differences in the total amount released as well as in the proportions within the volatile blend within each pair of cultivar. Hence, it remains to be elucidated whether the qualitative and quantitative differences found here for the Bt176-plants and their corresponding isogenic lines after damage by three different insect species as well as within the pair of cultivars Nobilis/ Novelis (MON810) might affect the attractiveness of the plants to parasitic wasps. Further experiments should be conducted with the parasitoids *Cotesia marginiventris* or *Microplitis rufiventris* to clarify this question. Even though the attractiveness of the transgenic plants is not diminished, there could be other effects by which the parasitoids are impaired. For example, by parasitizing hosts that have a decreased fitness due to the Bt-toxin or due to a reduced food quality of the food plants, the parasitoids might be

indirectly affected (see http://www.biosicherheit.de/mais/308.doku.html and Schuler et al., 1999).
Although a lot of studies were conducted on possible effects of the Bt-toxin on target and non-target herbivores or on the question whether carnivores and parasitoids might be indirectly affected by a reduced fitness of their host or prey (e.g. Hilbeck et al., 1999; Zwahlen et al., 2000; Hilbeck, 2001; Jesse and Obricky, 2004; Losey et al., 1999; Stapel et al., 1997; Schuler et al., 1999; Atwood et al., 1997; Ludy and Lang, 2006), only little information is available on both short-term and long-term effects of transgenic corn plants in the field. Ma & Subedi (2005) demonstrated for several Bt-cultivars a prolonged development until maturity and a similar or lower grain yield relative to their isogenic lines under field conditions. Furthermore, there was no yield advantage of Bt-lines in comparison to their isogenic cultivar when the infestation with *O. nubilalis* was low to moderate. This could also be confirmed by Catangui and Berg (2002) in another field study. In both reports, a higher grain moisture at harvest, which can result in moisture penalty or dockage, was found in the Bt-cultivars relative to their isolines (see also Dillehay et al., 2004). However, no information is available on the influence of the introduced Bt-gene on the volatile release of corn plants in the field. To examine the emission of transgenic plants in the field and whether the tritrophic system might be disturbed under these conditions, the corn lines analyzed in the laboratory were cultivated for three years in two field sites and the volatile release was collected. Moreover, the parasitation rates of larvae of the noctuid species *S. littoralis* were observed.
In principle, all volatiles found in the laboratory experiments for each cultivar were also found in the field. The only exception was β-sesquiphellandrene that was only released by corn plants in the laboratory. In general, the differences in the field between transgenic plants and their corresponding isogenic lines were not as pronounced as in the laboratory since the release of only few compounds was significantly different between Bt- and non-Bt plants. For instance, the pair of cultivar Prelude/Valmont (Bt176) showed significant differences between transgenic and isogenic line for three compounds, β-selinene, (*E*)-β-caryophyllene, and β-myrcene, but only in certain months and/ or years. In the pair of cultivars Navares and Antares, significant differences between transgenic and isogenic corn line could be detected for DMNT and δ-cadinene over the whole season in 2003. Nevertheless, between transgenic and isogenic maize lines, no differences in overall emission were found over the years. The individual volatiles showed within the lines as well as between years or months incoherent patterns.
However, as it was shown already with laboratory data, the pair of cultivar Nobilis and Novelis (Mon810) exhibited the most pronounced differences in the volatile profile between a transgenic and a isogenic line also in the field. There were significant differences between both lines found for nine out of fourteen volatiles and the total amount emitted either among the complete field season like for limonene, or in dependence on the sampling date, i.e. a certain month like in case of DMNT or a certain year like for α- and β-selinene. Most striking has been the absence of one sesquiterpene, α-ylangene, in the blend of the transgenic plants, which was always found in the isogenic line. In general, the qualitative and quantitative volatile emission pattern of the transgenic cultivar was differently influenced by the time of the year than the isogenic line. The comparison of three consecutive years showed different pictures with regard to differences between the isogenic and transgenic lines. For example, the isogenic line emitted equal amounts in the years 2001 and 2003 and lower amounts in 2002, while the transgenic line showed the lowest emission in 2002 and the highest in 2003. Overall these results indicated that differences in the volatile blends between isogenic and transgenic lines exist in the field but with strong time dependencies.

Nevertheless, the factor time seemed to play a very important role in the variation of volatile emission patterns in the field. However, beside developmental features this is influenced mainly by abiotic factors such as high temperature, elevated ozone concentrations or water deficiency (Gouinguene and Turlings, 2002; Yatagai et al., 1995; Llusià and Peñuelas, 1998; Peñuelas et al., 1998; Llusià et al., 2002;Vercammen et al., 2001; Vallat et al., 2005). In the maize lines derived from the Bt-event Bt176, a similar trend was found regarding the volatile release over the years such that the emission increased from 2002 to 2003. It was discovered by Loreto et al. (1998) that terpene synthesis dramatically increases at high temperatures and that fumigation with some monoterpenes increased the thermotolerance of the monoterpene-emitting oak *Quercus ilex* (see also Peñuelas, J. & Llusià, J., 2002). This led to the hypothesis that the plants might be protected from high temperatures by terpene emission (Singsaas, 2000). This fits nicely to the high overall emissions in the field in 2003, since in this year the maize plants were exposed to extraordinarily high day and night temperatures. The emission within the years, however, differed depending on the cultivar and in case of Prelude/Valmont also on the year. Although there were some differences between transgenic cultivar and their corresponding isogenic line, this effect was generally by far smaller when compared to the effect of sampling date, i.e. month or year. This implies that the temporal effect was much larger than the effect of the Bt-event Bt176.

Even though more differences between the transgenic and isogenic cultivars were found in the laboratory experiments, the field data do not contradict the results found in the laboratory. Laboratory experiments provide informations on the processes the plants can potentially perform, but responses of plants in the field are subjected to combinations of numerous factors such as exposure to fungi, herbivores or viruses as well as temperature, relative humidity, ozone-concentrations, or application of pesticides (see also Hern and Dorn, 2003 and Vallat et al., 2005) which might mask the effect of varieties. As discussed above, the emission of individual volatile compounds by transgenic maize plants is not only directly influenced by the transformation with a Bt-coding gene but might also be subjected to a certain time of the year in concert with the developmental stage of a plant (see Köllner et al., 2004) maybe in combination with abiotic factors or a certain year depending on climatic conditions (e.g., Hern and Dorn, 2003; Vallat et al., 2005; Llusia et al., 2002). Consequently, the volatile release in the field by transgenic corn plants cannot be predicted and thus should be observed over more years to evaluate the effects of climatic conditions on the performance of transgenic plants. The authorization of both Bt-events for commercial cropping should be reconsidered, in particular in case of MON810 since plants of this event are impaired in their ability to produce and/ or emit volatile compounds and furthermore show a reduced food quality as discussed above. Whether and to what extend the endogenous Bt-toxin in the transgenic plants does interfere with the tritrophic system under field conditions cannot be elucidated by the present study since the parasitation rates could not be specified. Even though in 2003 a lot of parasitic wasps were sighted in both fields, no parasitized larvae of the noctuid species *S. littoralis* were found. This might have several reasons. Probably there was no parasitoid community established in the field, which was attracted by the volatile pattern of corn induced by *Spodoptera*-feeding. This contradicts the personal observations that parasitic wasps were found equally among all corn lines in nearly 50 % of the cages. Since these wasps were perished and showed no preference for the isogenic over the transgenic cultivars, it might rather be possible that they were equally attracted by the volatile blend of all corn lines but could not parasitize the larvae of *S. littoralis*. Although some studies confirmed that the tritrophic interaction also exists in nature (e.g. Drukker et al., 1995; Kessler and Baldwin, 2001; Heil, 2004) and this indirect defense was furthermore found

for maize plants under field conditions (de Moraes, 1998), the ecological relevance in our latitudes is not yet proved. As mentioned in the introduction of this chapter, there are native predators as well as imported parasitoids in the United States, which may kill up to 30 % of the populations of the European corn borer in the field. Though, there is no information available, whether these predators and parasitic wasps might also affect populations of non-target lepidopterans such as *Agrotis segetum* or whether these or other antagonists play a role in the corncroppings in Europe.
Most remarkably, the volatile profile of all examined varieties showed the same unexpected behavior in the years 2002 and 2003 such that the volatile blend changed qualitatively at the end of the field season. Another field study also demonstrated a qualitative change in the volatile composition of apple trees and could correlate this to drought (Vallat et al., 2005), but contrary to the present study only one compound - the monoterpene camphene - was additionally emitted while the composition of the other components did not change. In the present field study, however, a more drastic change was observed: the whole sesquiterpene-fraction was not present anymore and the two sesquiterpenoids β-selinene and α-selinene, which were not yet described for commercial corn cultivars, were released, whereas the composition of the mono- and homoterpenes did not change. Interestingely, this complete change was found in all cultivars and in both field sites indicating a more comprehensive phenomenon. The question now arises what both selinenes are induced by and what might be the biological or ecological function of these compounds.
Since only in 2002 and 2003 an infestation of the corn plants with the European corn borer *O. nubilalis* was observed in both field sites and among all cultivars, which somehow corresponded to the presence of both selinenes in these years, this could be a possible reason for the induction of β-selinene and α-selinene. Several studies demonstrated that different feeding habits of herbivores could influence the release of volatiles. Takabayashi et al. (1995) found considerable qualitative differences between maize plants, which were attacked by young and old *Pseudaletia separata* caterpillars. Turlings et al. (1998b), however, reported only on quantitative differences in the volatile blend of maize seedlings damaged by the folivorous caterpillar *S. littoralis* or the stem borer *O. nubilalis*. As discussed above, there were qualitative differences in the volatile profile after infestation of corn plants with the herbivores *O. nubilalis*, *A. segetum* and *S. littoralis* but in neither treatment β- or α-selinene was found. However, it is difficult to mimic the feeding habit of *O. nubilalis* in the laboratory since it starts to damage the shoots and then continuously feeds inside the corn stalk until maturity of the host plants. For this reason, it cannot be excluded that these sesquiterpenoids can be induced by a long-term infestation by *O. nubilalis* and might function as defensive compounds. Momin et al. (2000) reported on β-selinene to be toxic for *Aedes aegyptii* larvae with a 100 % mortality at a concentration of 50 µg/ml and nearly 40 % mortality at 12.5 µg/ml. Hence, it should be tested in bioassays whether β- and α-selinene might interfere with larval development of lepidopteran herbivores.
Throughout the field seasons 2001 to 2003, the corn plants were exposed to climatic conditions that changed within the growing season as well as between the years. Whereas in 2001 the climatic conditions were somehow in the range usually found in middle Europe, in 2002, a dry and warm summer was followed by a short period of hard rain and flooding. During July and August 2003, however, the plants experienced drought and extraordinary high temperatures, and furthermore increased ozone concentrations for a longer period of time. Since these climatic changes were present in both field sites, the emission of the sesquiterpenoid compounds β- and α-selinene might have been induced by abiotic factors such as high temperature, drought, waterlogging or elevated ozone

concentrations. Numerous studies in the laboratory as well as under field conditions demonstrated the dependence of volatile release on these abiotic factors. A study on corn seedlings showed that temperatures between 22 °C and 27 °C seemed to be optimal for a higher emission of herbivory-induced volatiles compared to lower or higher temperatures but no qualitative change was observed (Gouinguene and Turlings, 2002). Also, plants under water deficiency, a stress that is often correlated with heat stress, can be affected in their ability to produce volatiles. In Mediterranean wood species, the terpene emission rates severely decreased under drought conditions (Llusia and Peñuelas, 1998). Recently, a field study in an apple orchard in 2003 showed that the emission of certain volatiles from apple trees negatively correlated with rainfall, thus supporting the theory that drought might cause a higher synthesis of secondary metabolites (Vallat et al., 2005). For the opposite stress, the hypoxia or waterlogging, little information is available on the effects on volatile emission. Gouinguene and Turlings (2002) showed a decrease in volatile emission if corn seedlings were exposed to 80 %-saturated soil but no qualitative change in the volatile profile. Also ozone was demonstrated to influence the volatile emission of plants. Recently, Vuorinen et al. (2004) reported on lima bean that both ozone-exposure and spider-mite infestation induced the release of volatiles attractive to predatory mites. The authors found that the mites were equally attracted to unexposed and ozone-exposed plants, but could discriminate between uninfested plants and spider-mite-infested plants when both were previously treated with ozone. According to Schulze et al. (2002) maize plants belong to the relatively sensitive organisms to ozone, which can be damaged at a concentration above 0.2 ppm. Nevertheless, whether and to what extend the volatile release of corn plants might be impaired by ozone-exposure, is not known. Since the climatic conditions near both field sites as well as directly in the field sites are known, it should be possible to mimic the abiotic factors high temperature, drought, waterlogging, and ozone exposure and investigate whether one of these factors might have been responsible for the drastic change in the volatile blend at the end of the field seasons 2002 and 2003.

As demonstrated in table 2.2, various herbicides were applied in the field near Halle in all three field seasons. Little information is available on whether and how the application of pesticides to a plant interferes with its volatile emission. Vercammen et al. (2001) demonstrated the release of several isothiocyanate compounds by *Arabidopsis thaliana* plants upon spraying with Paraquat. More recently, Vallat et al. (2005) reported on a qualitative change in the volatile profile of apple trees and argued that among abiotic factors also the application of pesticides might change the composition of the volatile blend. Consequently, the induction of β-selinene and α-selinene also might be a result of the exposure of the maize plants to a herbicide.

3. The role of maize terpenes in direct defense against insect herbivores

3.1. Introduction

3.1.1. Direct defense of plants

Kessler and Baldwin (2002) defined direct defenses as "any plant traits… that by themselves affect the susceptibility to and/ or the performance of attacking arthropods", leading to an increased fitness of the attacked plant. This includes morphological adaptations such as thorns, silica or trichomes as well as the accumulation of primary or secondary metabolites. Unlike primary metabolites, secondary metabolites are not directly involved in growth and development but often play a role in plant defense. For example, proteinase inhibitors or antidigestive proteins are inducible by wounding and herbivory and influence the herbivore by inhibiting insect digestive enzymes (Tamayo et al., 2000). Polyphenol oxidases are enzymes which decrease the nutritive value of the wounded plant by cross-linking proteins or catalyzing the formation of reactive or polymerizing quinones. Toxins such as alkaloids, terpenoids or phenolics, can poison generalist herbivores and force specialist herbivores to invest resources in detoxification mechanisms detracting energy from growth, development and reproduction (Kessler and Baldwin, 2002). Terpenoids represent the largest group among secondary metabolites and interfere with insect growth or development in numerous ways. The toxicity of many terpenes to microbes and insect herbivores indicates that they are involved in direct plant defense (Gershenzon and Croteau, 1991). The terpenoid *cis*-nerol for instance, was found to be highly toxic to the Formosan subterranean termite *Coptotermes formosanus*, leading to morphological abnormalities in the exoskeleton after exposure (Zhu et al., 2003). A study on three stored-product beetles demonstrated contact and fumigant toxicity of D-limonene to the larvae of the tested species (Tripathi et al., 2003). Furthermore, these authors could also show a feeding deterrence of D-limonene on all three beetle larvae and a decrease in oviposition rate of one beetle species. Consequently as deterrents, terpenoids can prevent feeding or oviposition of insects. Moreover, some terpenes mimic insect hormones and thereby can negatively affect insect development and growth. This was found by Mauchamp and Pickett (1987) for (*E*)-β-farnesene, the aphid alarm pheromone, which showed a comparable activity to juvenile hormone. In grand fir (*Abies grandis*), it was demonstrated that sesquiterpenes based on a bisabolane skeleton mimicked juvenile hormones and thus, could interfere with insect development and reproduction (Bowers et al., 1976).

3.1.2. Volatile emission of maize

The volatile composition of maize is well investigated and has been recorded for many different cultivars. Remarkable quantitative and qualitative differences in herbivore-induced volatile blends were found among different maize cultivars and in comparison to the wild ancestor theosinthe (Gouinguene et al., 2001). Furthermore, Köllner et al. (2004) showed that sesquiterpenes as the major components in the maize blend differ to a large extent among different organs and developmental stages.
Figure 3.1. shows the structures of eleven terpenoid compounds that have been found in the cultivars examined in this study (chapter 2) and are emitted in response to herbivore

infestation, for example (*E*)-β-farnesene and (*E*)-β-caryophyllene, or constitutively in old corn plants, such as β-bisabolene and δ-cadinene (see also Köllner et al., 2004).

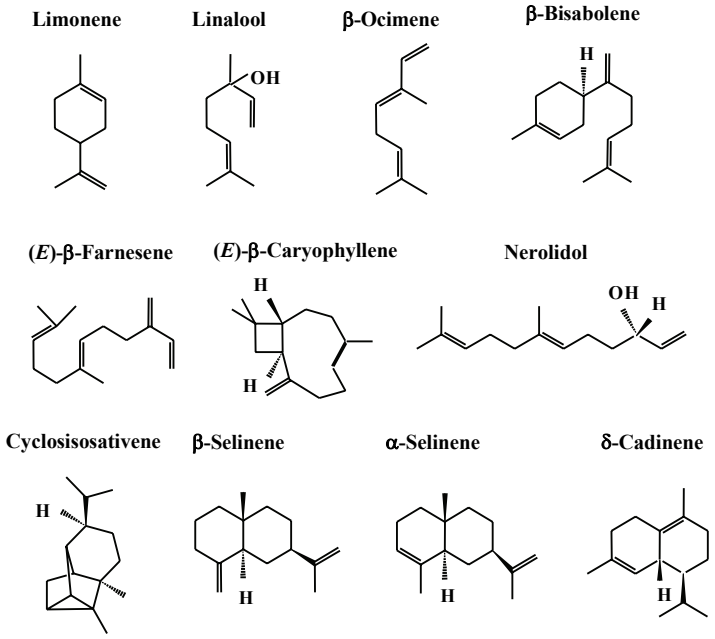

Figure 3.1. Chemical structures of mono- and sesquiterpenes examined in this study

3.1.3. *Spodoptera littoralis* (Boisduval) as model insect

The egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) belongs to the family Noctuidae and is distributed in the Mediterranean region and in Africa. This species and other species from the genus *Spodoptera* belong to the major pest insects on tomato and cotton, especially in Egypt where they can generate up to 10 generations per year. In central Europe, *Spodoptera* species are categorized as quarantine organisms, because they also infest green houses. Caterpillars of this genus are extremely polyphagous; more than 100 plant species from 44 families can be used as host, with 87 of these species being important as crop plants. Because some populations of *S. littoralis* are resistant against insecticides, these pest insects are extremely hard to control. The 0.6 mm-long eggs (figure 3.2a) are deposited by the female moth in huge amounts on a host plant and covered with hairs from the abdomen. After hatching, the larvae are 2-3 mm long, white colored, and live gregariously (figure 3.2a and b). Older larvae turn darker with a dorsal pattern (figure 3.2c and d) and live more solitarily and nocturnally. Full-grown larvae can be up to 4.5 cm long after passing through five to six instars. Right before pupation, the larvae carve 2-5 cm burrows in the soil. From the auburn, approximately 2 cm long pupae (figure 3.2e), moths with a length of 2 cm and a wing span of 4 cm emerge, whose wings are brown colored with a bright pattern (figure 3.2f).
Species of the genus *Spodoptera* are often used in scientific examinations due to their high grade of polyphagy and their relatively simple cultivation on artificial diets, e.g. on basis of soy or beans. Because cannibalism in young larval stages is rare, it is quite easy to cultivate them in large numbers. In addition, they are good subjects for bioassays and feeding tests since they seem to possess diverse detoxification mechanisms and thus are not very sensitive to pesticides. If plant chemicals have an effect on *Spodoptera*-larvae, it seems to be likely that this effect can also be found in other, more sensitive pest insects.

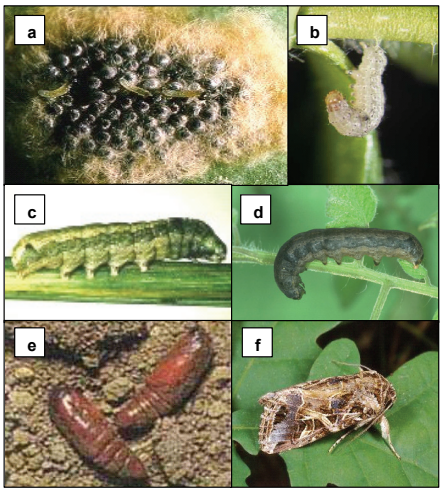

Figure 3.2. Different life stages of *Spodoptera littoralis* (Boisd.)
a: egg clutch with newly hatched larvae; b: small larvae; c: old larvae, bright form; d: old larvae, dark form; e: pupae; f: adult moth (public domain photographs)

3.1.4. Scope of this chapter

Terpenes are widespread and well investigated plant secondary metabolites that are often associated with interactions of plants with insects. In many studies it has been shown that herbivory by lepidopteran larvae can induce the formation of complex mixtures of volatile terpenes which serve to attract parasitic wasps that use the larvae as hosts (e.g. Dicke et al., 1990; Llusia & Penuelas, 2001). Moreover, for many terpenoids it was shown that they are bioactive against insects (e.g. Zhu et al., 2003; Tripathi et al., 2003) but it is still unclear if the terpene mixtures in maize can also serve in direct defense against herbivores as toxins or feeding deterrents. To test their effects individually on larval development of *Spodoptera littoralis*, several mono- and sesquiterpenes that are available as pure standards and naturally occur in the volatile composition of corn plants were chosen (chapter 2 and Köllner et al., 2004). As reported by Momin et al., 2000 β-selinene was toxic to mosquito larvae. To examine whether the emission of β- and α-selinene in the field in 2002 and 2003 might be an activated direct defense against *Ostrinia nubilalis*, these sesquiterpenes were isolated from celery oil and tested for their effects on larval development of *S. littoralis*.

3.2. Materials and Methods

3.2.1. Insects

All feeding tests were conducted with the Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.). Eggs were obtained from Syngenta (Basel, Switzerland) and reared on an artificial bean diet (Inga Mewis, personal communication). To a mixture of 500 g of bean flour (Huber Mill; Hohberg, Germany) and 500 ml tap water, 9 g ascorbic acid (ROTH; Karlsruhe, Germany), 5 g ethyl 4-benzoic acid (Aldrich 19128-0; Steinheim, Germany) and a mixture of 0.6 ml α-tocopherol (FLUKA; Buchs, Switzerland) and 9 ml Mazola germ oil (Unilever Bestfood, Hamburg, Germany) were added. To prevent insect diseases and spoiling of the diet, 4 ml of 3.7 % formaldehyde (37 %; Roth) were added. 300 g of

diet were thoroughly mixed with 200 ml of 7.5 % agar solution and poured into 600 ml plastic containers. This diet can be stored for at least three months at 4 °C.

3.2.2. Bioassay

The terpenes tested as well as their concentrations are listed in table 3.1.
All feeding tests were conducted with an artificial diet. For each tested compound 1 g agarose (Bio-Rad; Hercules/ California, USA) was added to 50 ml tap water and heated until the agarose was completely dissolved. The clear agarose-solution was mixed with 8 g finely ground frozen maize leaves from the cultivar Prelude and 2 g bean flour, and then stored in a water bath at 50 °C. To prevent infection by fungi or bacteria, 50 µl of 3.7 % formaldehyde (Roth) were added. Terpene standards were diluted with dimethyl sulfoxide (DMSO, 99.8 %; Roth) so that the volume of DMSO with terpene standard did not exceed 20 µl for high terpene concentrations and 2 µl for low terpene concentrations in a 50 ml diet. The same amounts of pure DMSO were added to the control diet. After mixing all components, the diet was distributed with a 5 ml pipette into ten 4 ml HPLC vials, the weight of the filled vials was recorded, and each vial was placed in a Petri dish. The diet was prepared twice for each terpene and control in order to test 20 caterpillars from the same egg clutch per treatment with two different batches of diet.

Table 3.1. Terpenes and concentrations tested in the feeding tests

Compound	Purity (%)	Feeding test and concentration (µg/g diet) 1	2	3	4	5	6
α-selinene	**58.3**						0.011 & 17.000
β-bisabolene	**99.0**					0.0018 & 1.800	
(*E*)-β-caryophyllene	**99.0**	0.178	4.000	40.000			
(*E*)-β-farnesene	**99.0**	0.270	4.000	40.000			
β-myrcene	**97.0**	0.001	3.400	34.000			
(*E*)-β-ocimene	**97.0**	0.030	4.900		49.000		
β-selinene	**83.1**						0.017 & 18.000
cycloisosativene	**99.0**	0.037			36.800		
δ-cadinene	**97.0**	0.009		3.600			
limonene	**90.0**	0.152	3.300	33.400			
linalool	**95.0**	0.410			36.800		
nerolidol	**98.0**	0.0035	3.900	39.000			

Ten to twelve days after hatching, the larvae were placed on agarose gel diet without terpenes and DMSO during two days to adapt to the new diet. On the day the experiment started, larvae with a weight between 13 and 30 mg were chosen and distributed equally among all treatments and control so that the starting weight of the groups did not differ significantly. In all feeding tests 20 caterpillars were tested per treatment and control in two independent replications with 10 caterpillars each replication. During the experiment, all Petri dishes were stored for one week in a climate chamber with a 8 h: 16 h dark-light regime, relative humidity of 70 % and temperature of 22 °C to ensure that all experiments were conducted under the same climatic conditions. To estimate the weight loss of diet due to evaporation, five additional vials with control diet were prepared and stored under the same conditions but without caterpillars. After seven days, the weight of the remaining diet was measured and the diet was replaced with the same diet without terpenes or DMSO. Caterpillars were weighed individually every day until pupation.

3.2.3. Availabilty of terpenes in the agarose-diet

To examine whether the terpenes added to the diet can be recovered and to what extent they evaporate, the emission of terpene volatiles from the diet was measured. Six 5-ml-aliquots of the terpene-containing diet were pipetted into six 20 ml-test-tube and were allowed to solidify. A trap filled with SuperQ (Alltech) and a teflon-tube were placed on the top of each test-tube which was then closed with teflon-tape to prevent contaminations. Filtered air was applied with a flow-rate of 2 l/min to the glass-vials via the teflon-tube and pumped with a flow-rate of 1.5 l/min through the trap to allow the absorbance of the emitted volatiles. After a 6-hour-measurement, the volatiles were eluted from the traps with 200 µl pentane containing an internal standard (nonyl-acetate, 40 ng/µl) and subsequently analyzed by GC-MS. These measurements were conducted on the next seven days after preparing the diet.
Additionally, the amount of terpenes in the diet was tested using the following procedure: 50 g of the diet were prepared as described in chapter 3.2 and each standard was added to the same final concentrations as they were used in the bioassays in the lowest concentrations (see table 3.1). Directly after the diet was cooled down and solidified in a glass bottle, 25 g of diet were hackled, transferred to another glass bottle and 50 ml of hexane were added. This mixture was incubated at 4 °C for one week. After one week, the hexane was removed from the diet-hexane-mixture, the volume reduced to 2 ml by evaporation and 50 ml of fresh hexane were added to the diet for one additional week. After this incubation time, the 50 ml hexane were removed, reduced to a sample size of 2 ml and both samples were pooled, resulting in a 4-ml-sample representing the terpene content directly after preparing of the diet. The remaining 25 g of the diet were stored for one week in a climate chamber (chapter 3.2) to mimic the conditions during the bioassay. This diet also was washed twice with hexane following this protocol, showing the terpene content in the diet after seven days. Alpha- and β-selinene were tested separately after their isolation.

3.2.4. Analysis of larval development

The following parameters were measured during the development of the larvae (see figure 3.3): weight at the beginning of the experiment in mg (*start weight*), weight on day seven of the experiment in mg (*end weight*), diet consumed during the experiment in g (*diet consumed*), highest weight measured in mg (*maximum weight*), day of pupation (*pupation*), duration of pupation until emergence of the moth in days (*duration of pupation*) and the day the moth emerged (*developmental time*).
To calculate the *diet consumed*, the weight loss due to evaporation was estimated for the five vials with control diet and the average percentage of weight loss was used for further calculation. The weight in the vials on day seven of the experiment was subtracted from the weight of diet on day one. From this remaining diet the percentage of weight loss due to evaporation was calculated and subtracted, resulting in the weight of diet consumed.
Statistical analysis was performed with the program SigmaStat 2.03 (SPSS Inc.). To compare arithmetic means, a t-test with independent samples was conducted. If the assumption of normal distribution and variance homogeneity of the different groups was not achieved, a U-test was used. Graphs were created with the program SigmaPlot 7.0 (SPSS Inc.), showing arithmetic means and standard error if not indicated otherwise.

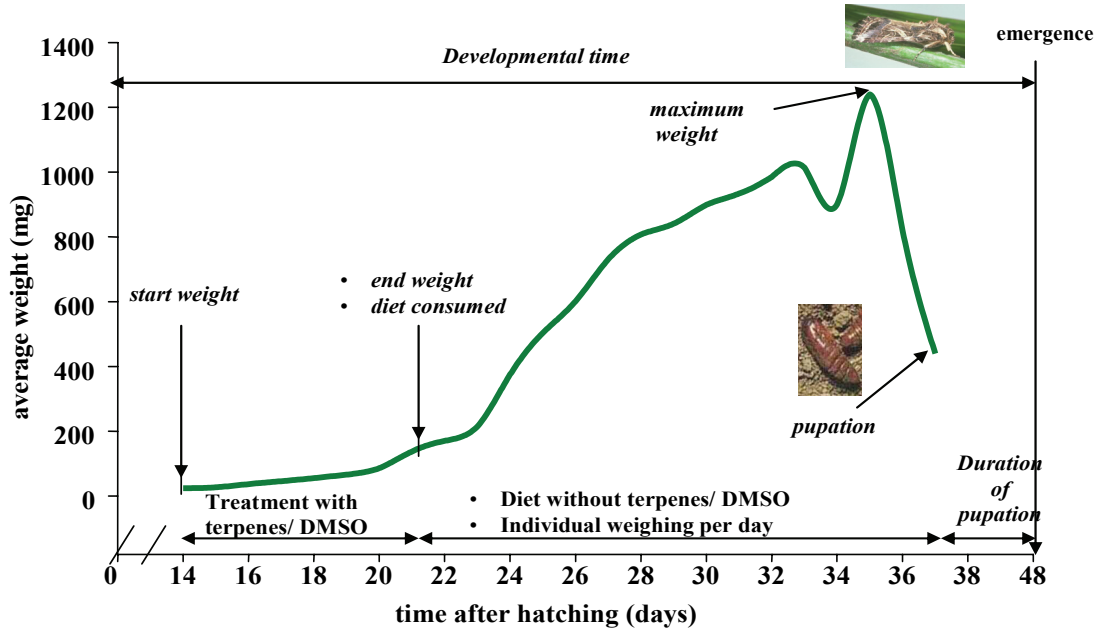

Figure 3.3. Procedure of a feeding test

3.2.5. Isolation of α- and β-selinene

As no pure selinene standard was available, celery oil was used for isolation of α- and β-selinene. The main constituent of celery oil is limonene making up ~ 70 % of the total oil, whereas β-selinene and α-selinene comprise ~ 20 %. Other minor constituents are (*E*)-β-caryophyllene, (*E*)-α-bergamotene and δ-cadinene (Σ: ~5 %).
The monoterpene limonene was removed from the sesquiterpene mixture because of its lower boiling point by heating the oil in a rotary evaporator (R-114; Büchi, Switzerland) for 3-5 h at 90 °C and 20 mbar (diaphragm vacuum pump R-114; Büchi). To eliminate minor sesquiterpenes, the remaining oil was separated by flash chromatography according to Still et al (1978). This method is an air pressure driven hybrid of medium pressure and short column chromatography which allows the separation of samples up to few grams in a relatively short time.
A glass column with a diameter of 40 mm was filled with a 12-cm-layer of dry 40-63 µm silica gel (Merck; Darmstadt, Germany) (see figure 3.4). On top of that a 2-cm-layer of 50-100 mesh sand was added to protect the surface of the silica bed. The glass column was carefully filled with 2 l *n*-hexane (Roth) before air pressure was applied to pack the silica gel. Two milliliters of celery oil from which limonene had been removed were evenly applied with a Pasteur-pipette on the top of the silica column and allowed to absorb into the silica gel by gravity. A few milliliters of fresh solvent were applied to the walls of the column to assure that the entire sample was absorbed by the gel, before the column was carefully refilled with the eluent. The column was eluted with 2 l of *n*-hexane by application of a constant air pressure that equals the pressure of a 1 m water column (figure 3.4). Fractions of 10 ml were collected in glass vials. The fractions were analyzed for the presence of separated compounds by Thin Layer Chromatography (TLC). Spots (~5 µL) of each fraction were applied along the long side of a 20 x 10 cm TLC plate (TLC aluminum sheets silica gel $60F_{254}$; Merck) and the TLC plate was developed in *n*-hexane. Afterwards the TLC was sprayed with a *p*-anisaldehyde solution (5 ml anisaldahyde in 5 ml 95 % H_2SO_4 and 95 ml EtOH) and heated for 1-2 min at 120 °C. Fractions that were

stained were further analyzed by GC-MS and those fractions containing selinene were used for further purification steps.

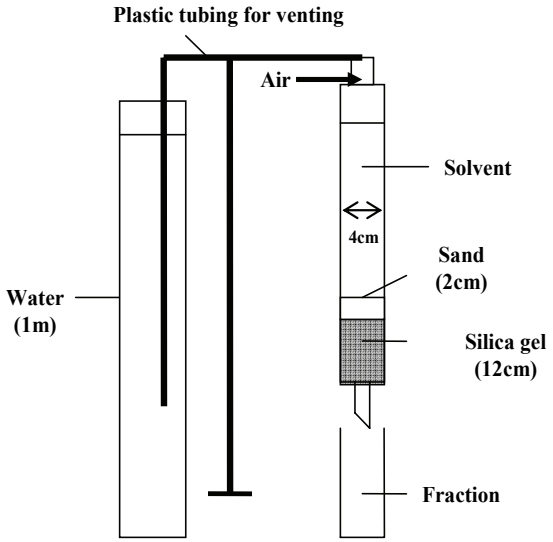

Figure 3.4. Experimental setup for flash chromatography

Since β-selinene, α-selinene and β-caryophyllene were present in the same fractions, an additional purification step using argentation chromatography was necessary. To prepare the column material, 3 g of silver nitrate (Merck) were dissolved in 30 ml distilled water and mixed with 40-63 µm silica gel (Merck) in a flask covered with aluminum foil since silver nitrate is sensitive to light. This mixture was dried for 1.5 h in a oven at 150 °C and left overnight under vacuum in a dessicator over P(V)-oxide. The dried mixture was filled in a glass column (30 mm diameter) covered with aluminum foil and topped with 2 cm of sand. A mixture of 90 % *n*-hexane and 10 % ether (inhibitor free; Sigma-Aldrich) was used as eluent. Packing of the column, loading of the sample and separation was the same as described above. The fractions were analysed by TLC and GC-MS. Since silver nitrate interacts with double bonds, β-selinene which contains two exocyclic double bonds was retained to a higher degree by the column than β-caryophyllene with one exocyclic double bond, and eluted in later fractions with more than 80 % purity (figure 3.5).

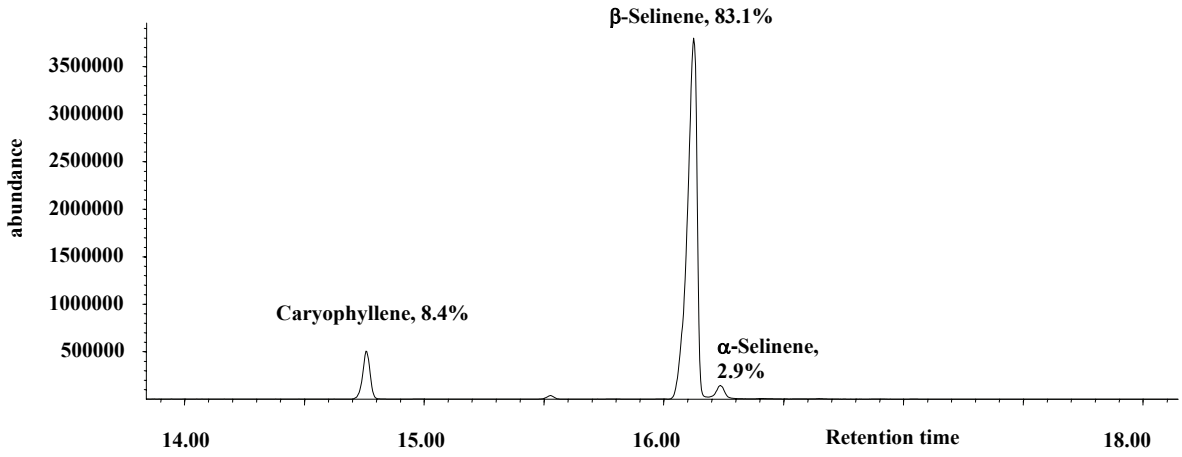

Figure 3.5. Fractions 9-12 with isolated β-selinene

Alpha-selinene with one exocyclic double bond was present in fraction 3 at almost 60 % purity (figure 3.6).

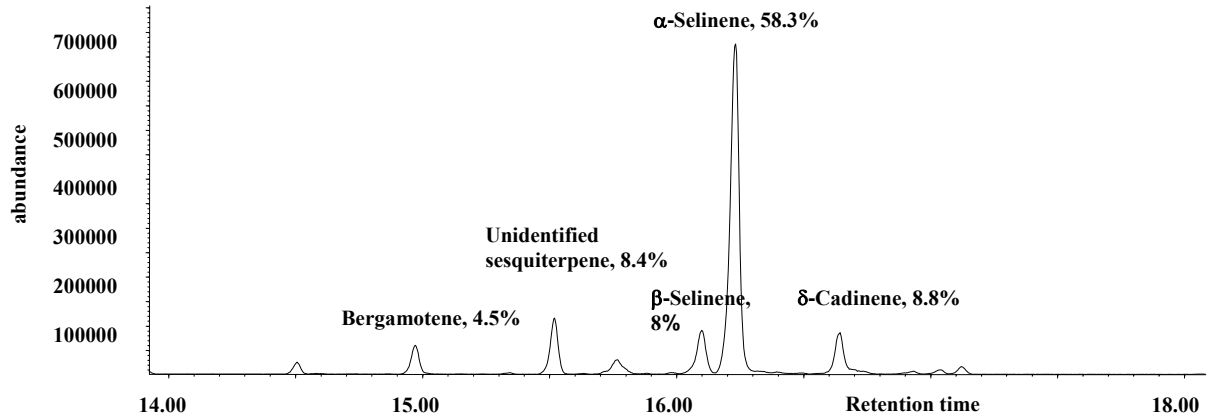

Figure 3.6. Fraction 3 with isolated α-selinene and several minor components

3.3. Results

3.3.1. Availability of terpenes in the agarose-diet

To determine the amount of terpenes that evaporate from the diet, daily volatile measurements were conducted for one week. During all measurements, none of the terpenes could be detected in the samples (see figure 3.7), indicating that the terpenes are not released as volatiles in detectable amounts from this diet.
Furthermore, it was tested whether the applied terpenes could be regained from the agarose-diet directly after preparing and after one week. The extractions with hexane showed a 54-86% of recovery after one week depending on the terpene (table 3.2).
No significant differences in terpene concentrations were found between the extracts directly after preparation (data not shown) and after storage for one week in a climate chamber, indicating that most of the terpenes remain in the diet throughout the one-week feeding trial. A certain percentage of each terpene, however, seems to interact with the diet in a way that the terpenes can not be extracted completely by the solvent.

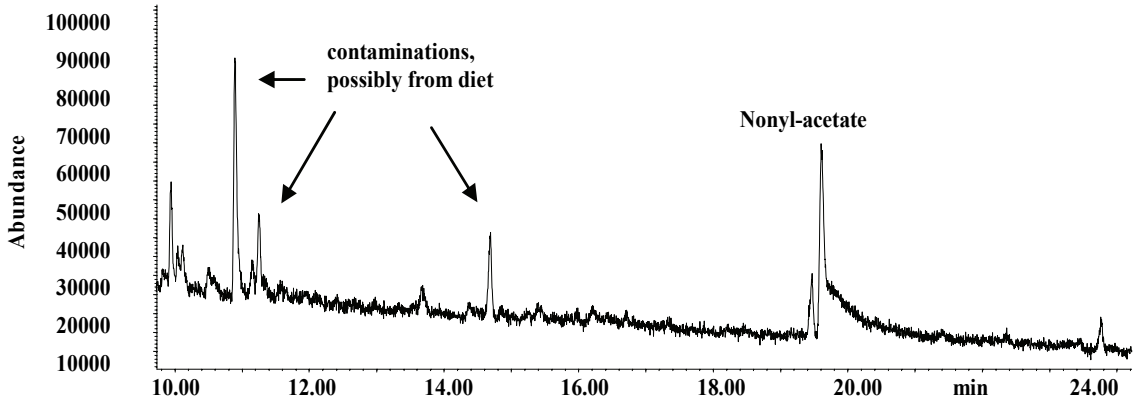

Figure 3.7. Volatile measurement on day seven after preparing the diet

Table 3.2. Concentrations of terpenes (ng/g diet), calculated and recovered after storing of the diet for one week

Compound	calculated concentration	recovered concentration
α-selinene	11.0	9.3
β-bisabolene	1.8	1.2
(*E*)-β-caryophyllene	178.0	96.7
(*E*)-β-farnesene	270.0	183.7
β-myrcene	1.0	0.7
(*E*)-β-ocimene	30.0	25.6
β-selinene	17.0	12.2
cycloisosativene	37.0	31.7
δ-cadinene	9.0	6.5
limonene	152.0	83.0
linalool	410.0	315.9
nerolidol	3.5	2.6

3.3.2. Feeding test 1

To test whether the sesquiterpenes that are emitted by maize plants play a role in the direct defense against lepidopteran herbivores, feeding assays with larvae of the generalist species *S. littoralis* were conducted on artificial diets containing pure terpenes. Here, the concentrations for herbivore-induced or constitutive terpenes were chosen that correspond to those determined for herbivory-induced or mature corn plants, respectively. In the first experiment, the following terpenes were tested (table 3.1): (*E)*-β-caryophyllene (178 ng/g diet); (*E)*-β-farnesene (270 ng/g diet); β-myrcene (1 ng/g diet); β-ocimene (30 ng/g diet); cycloisosativene (37 ng/g diet); δ-cadinene (9 ng/g diet); limonene (152 ng/g diet), linalool (410 ng/g diet), and nerolidol (3.5 ng/g diet). None of the pupae developed to butterflies, presumably due to the high humidity in the breeding container during the pupal stage. Hence, the parameters duration of pupation, developmental time and emergence success could not be estimated. An overview of the results of all examined parameters and terpenes tested in this experiment is shown in table 3.3. For limonene and β-myrcene there was no effect observed, whereas (*E*)-β-ocimene caused positive effects on larval development.

Table 3.3. Results of tested parameters in feeding test 1

Arithmetic means and standard errors are shown for the tested parameters end weight, diet consumed, maximum weight, pupation and mortality.

Ctrl: control; β-car: (*E)*-β-caryophyllene; β-far: (*E)*-β-farnesene; β-myr: β-myrcene; β-oc: (*E*)-β-ocimene; cyc: cycloisosativene; δ-cad: δ-cadinene; lim: limonene; lin: linalool; ner: nerolidol. Stars (t-test) and double cross (U-test) show significant differences compared to control (*/ $^{\#}$: p<0,05; **/ $^{\#\#}$: p<0,01; ***/ $^{\#\#\#}$: p<0,001); N = 20.

	ctrl	β-car	β-far	β-myr	β-oc	cyc	δ-cad	lim	lin	ner
End weight (mg)	90.7 ± 10.9	139.1** ± 9.4	91.7 ± 15.7	100.8 ± 6.0	132.3* ± 12.5	53.3$^{\#\#}$ ± 3.7	63.2* ± 12.8	92.1 ± 9.4	261.8*** ± 25.1	92.3 ± 10.5
Diet consumed (g)	0.6 ± 0.1	1.1** ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.3*** ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.85 ± 0.1	1.6*** ± 0.1	0.8 ± 0.1
Maximum weight (mg)	1358 ± 77	1249 ± 85	1353 ± 41	1394 ± 71	1352 ± 47	1424 ± 39	1396 ± 47	1378 ± 51	1270 ± 33	1307 ± 64
Pupation (day)	35.1 ± 0.5	35.1 ± 0.7	37.1$^{\#}$ ± 0.5	35.2 ± 0.3	33.4* ± 0.5	34.9 ± 0.2	35.3 ± 0.5	34.2 ± 0.6	30.8$^{\#\#\#}$ ± 0.5	33.6$^{\#}$ ± 0.5
mortality (%)	15.0	25.0	10.0	15.0	15.0	5.0	5.0	20.0	5.0	10.0

Larvae exposed to this compound showed higher end weight and diet consumed and the larvae pupated earlier than larvae reared on the diet without terpenes.Moreover, larvae treated with *(E)*-β-caryophyllene consumed more diet compared to the control larvae and were heavier after 7 days. Both, δ-cadinene and cyclosisosativene (figure 3.8) resulted in a lower end weight of the larvae after the treatment with terpenes than the control. *(E)*-β-farnesene treated caterpillars pupated two days later (figure 3.8), whereas the larvae that fed on nerolidol pupated 1.5 days earlier compared to the control group. The most striking effect could be found for linalool (figure 3.8): after the 7-day treatment the caterpillars consumed more diet and were almost three times heavier and pupated four days earlier than the control. None of the tested terpenes affected the maximum weight of the caterpillars.

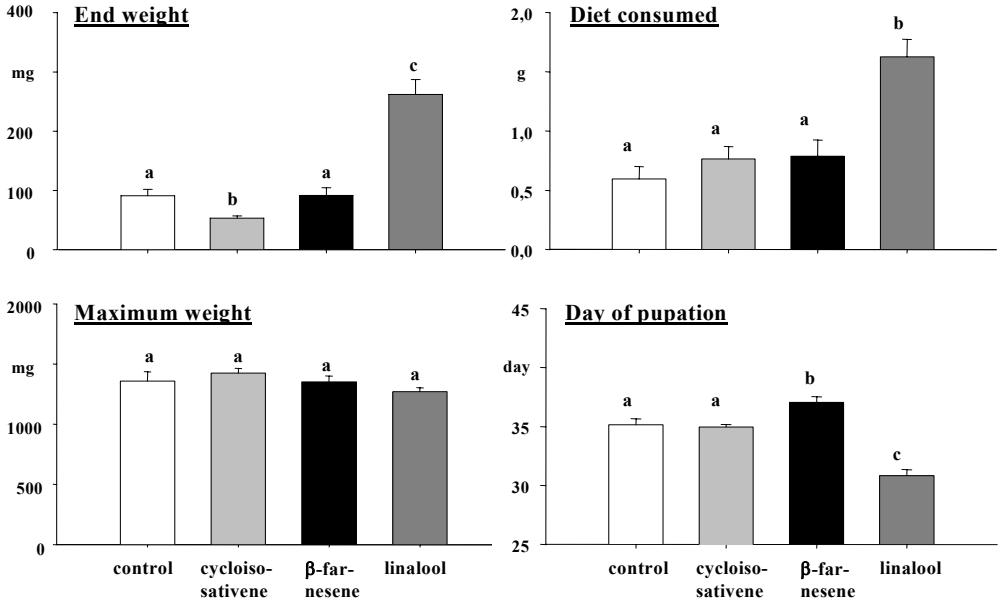

Figure 3.8. Feeding test 1

Arithmetic means and standard errors are shown for the parameters end weight, diet consumed, maximum weight and time of pupation. Results are shown for control, cyclosisosativene (368 ng/g diet), (*E*)-β-farnesene (27 ng/g diet) and linalool (410 ng/g diet). Significant differences compared to control are characterized by different letters; N = 20.

3.3.3. Feeding test 2

The second feeding experiment was conducted to supplement the results of the first. For this reason, the same terpenes were tested at higher concentrations (table 3.1): *(E)*-β-caryophyllene (4 µg/g diet); *(E)*-β-farnesene (4 µg/g diet); β-myrcene (3.4 µg/g diet); β-ocimene (4.9 µg/g diet); limonene (3.3 µg/g diet) and nerolidol (3.9 µg/g diet). An overview of the results of all examined parameters and terpenes tested is given in table 3.4. (*E*)-β-farnesene was the only terpene which did not affect significantly the examined parameters. In case of (*E*)-β-ocimene, the diet consumed and end weight of the larvae increased, while they pupated more than one day earlier (figure 3.9) compared to the control. Also for *(E)*-β-caryophyllene, the diet consumed and the end weight after treatment were higher than for control larvae (figure 3.9). Beta-myrcene and limonene showed an increased end weight after treatment in comparison to the control group. The maximum weight was not affected significantly by any of the treatments except for nerolidol (figure 3.9 and table 3.4). For nerolidol, the most remarkable effect on larval development was found (figure 3.9). Here, diet consumed and end weight were increased,

but the maximum weight of the larvae was lower and they pupated almost two days earlier than the larvae that were reared on control diet. In none of the treatments, however, did the duration of pupation, the developmental time or emergence success show significant differences relative to control (table 3.4).

Table 3.4. Results of tested parameters in feeding test 2
Arithmetic means and standard deviations are shown for the tested parameters end weight, diet consumed, maximum weight, pupation, mortality, duration of pupation, developmental time and emergence success. Ctrl: control; β-car: *(E)*-β-caryophyllene; β-far: *(E)*-β-farnesene; β-myr: β-myrcene; β-oc: (*E*)-β-ocimene; lim: limonene; ner: nerolidol. Stars (t-test) and double crosses (U-test) show significant differences compared to control (*/ $^{\#}$: p<0,05; **/ $^{\#\#}$: p<0,01; ***/ $^{\#\#\#}$: p<0,001); N = 20.

	ctrl	β-car	β-far	β-myr	β-oc	lim	ner
End weight (mg)	116.1 ± 9.7	167.4* ± 17.4	149.8 ± 17.6	145.8$^{\#}$ ± 8. 9	171.1** ± 15.0	180.0** ± 16.4	294.9*** ± 26.9
Diet consumed (g)	0.6 ± 0.1	0.9** ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9$^{\#}$ ± 0.1	0.7 ± 0.1	1.0*** ± 0.1
Maximum weight (mg)	1033 ± 43	1046 ± 46	976 ± 50	995 ± 55	921 ± 57	952 ± 40	857** ± 38
Pupation (day)	34.5 ± 0.4	34.0 ± 0.5	33.8 ± 0.4	34.9 ± 0.4	33.2** ± 0.4	34.0 ± 0.5	32.7$^{\#\#\#}$ ± 0.4
Duration (day)	14.1 ± 0.5	14.3 ± 0.4	15.0 ± 0.4	14.3 ± 0.5	15.4 ± 0.5	14.6 ± 0.5	15.5 ± 0.5
Developmental time (day)	48.6 ± 0.1	48.5 ± 0.2	48.2 ± 0.2	48.4 ± 0.2	48.3 ± 0.2	48.5 ± 0.2	48.4 ± 0.2
mortality (%)	10.0	10.0	10.0	0.0	5.0	5.0	5.0
emergence success (%)	78.0	83.0	78.0	75.0	68.0	68.0	75.0

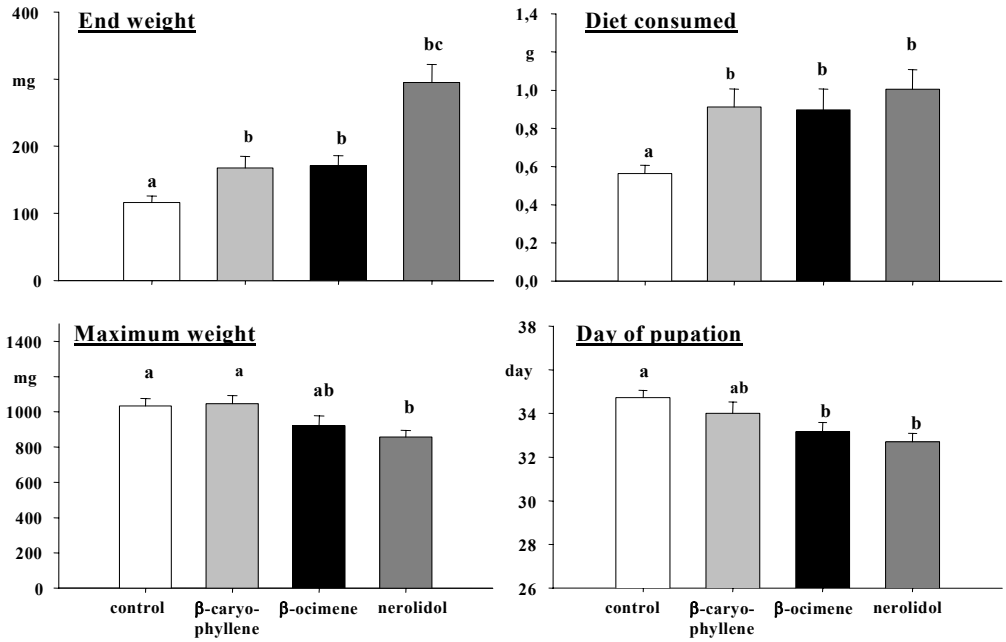

Figure 3.9. Feeding test 2
Arithmetic means and standard errors are shown for parameters end weight, diet consumed, maximum weight and time of pupation. Results are shown for control, (*E*)-β-caryophyllene (4 µg/g diet), β-ocimene (4.9 µg/g diet) and nerolidol (3.9 µg/g diet). Significant differences compared to control are characterized by different letters; N = 20.

3.3.4. Feeding test 3 and 4

These experiments were conducted to examine whether terpenes that showed no or little effects in low concentrations may influence larval development of herbivores in higher concentrations, and whether the effects found for several terpenes in lower concentrations could be enhanced with increasing concentrations. In experiment 3, the following terpenes were tested (table 3.1): (*E*)-β-caryophyllene (40 µg/g diet); (*E*)-β-farnesene (40 µg/g diet); limonene (33.4 µg/g diet); δ-cadinene (3.6 µg/g diet); nerolidol (39 µg/g diet) and β-myrcene (34 µg/g diet). In experiment 4, β-ocimene (49 µg/g diet); cycloisosativene (36.8 µg/g diet) and linalool (36.8 µg/g diet) were tested. An overview of the results of all examined parameters and terpenes tested in these experiments is given in table 3.5.
Due to unknown circumstances, the pupae did not develop to moths and the parameters duration of pupation, developmental time and emergence success could not be examined. For the terpenes (*E*)-β-caryophyllene, nerolidol, β-myrcene and δ-cadinene, none of the parameters examined was affected. In the group of larvae treated with *(E)*-β-farnesene, a significantly lower end weight and a delay of pupation could be shown compared to the control, but the other parameters were not significantly different. In the group which was treated with limonene, the caterpillars consumed more diet and were heavier after the 7-day-treatment, while no impact could be found for the other parameters.

Table 3.5. Results of tested parameters in feeding tests 3 and 4
Arithmetic means and standard errors are shown for the tested parameters end weight, diet consumed, maximum weight, pupation and mortality.
Ctrl: control; β-car: (*E*)-β-caryophyllene; β-far: (*E*)-β-farnesene; lim: limonene; δ-cad: δ-cadinene; ner: nerolidol; β-myr: β-myrcene; β-oc: (*E*)-β-ocimene; cyc: cycloisosativene; lin: linalool. Stars (t-test) and double crosses (U-test) show significant differences compared to the corresponding control (*/ $^{\#}$: $p<0,05$; **/ $^{\#\#}$: $p<0,01$; ***/ $^{\#\#\#}$: $p<0,001$); N = 20.

	ctrl	β-car	β-far	lim	δ-cad	ner	β-myr	ctrl	β-oc	cyc	lin
End weight (mg)	239.3 ± 21.3	264.8 ± 18.3	152.6** ± 4.1	311.7* ± 18.8	239.2 ± 20.0	241.2 ± 25.0	277.6 ± 20.9	304.6 ± 29.8	268.7 ± 2.9	233.6 ± 13.5	239.5 ± 24.2
Diet consumed (g)	1.4 ± 0.1	1.6 ± 0.1	1.2 ± 0.1	1.9* ± 0.2	1.7 ± 0.1	1.5 ± 0.2	1.6 ± 0.1	1.2 ± 0.1	0.8* ± 0.1	0.8** ± 0.1	0.8* ± 0.1
Maximum weight (mg)	689.1 ± 35.1	703.3 ± 49.7	738.1 ± 39.5	682.1 ± 39.5	747.7 ± 29.6	750.9 ± 49.4	695.9 ± 44.7	781.9 ± 35.3	799.0 ± 4.5	710.3 ± 32.9	692.3 ± 33.6
Pupation (day)	34.1 ± 1.0	33.8 ± 0.9	35.9* ± 0.8	34.3 ± 1.0	34.6 ± 0.6	34.0 ± 0.8	35.30 ± 0.7	31.9 ± 0.8	33.5 ± 0.7	33.0 ± 0.5	34.1* ± 0.6
mortality (%)	35.0	40.0	30.0	40.0	25.0	20.0	30.0	10.0	5.0	5.0	5.0

3.3.5. Feeding test 5

It has been previously shown that sesquiterpenes with a bisabolane skeleton can mimic insect juvenile hormones and thus can disturb insect development (Bowers et al., 1976). In another study, antifeedant effects of bisabolene on the Colorado potato beetle *Leptinotarsa decemlineata* were reported (Gonzalez-Coloma et al., 1995). To examine whether one of these effects could also be found for *S. littoralis* larvae, β-bisabolene was tested in two different concentrations (1.8 ng/g diet and 1.8 µg/g diet) and the terpene

containing diet was given either for seven days or until pupation. An overview of the results of all examined parameters in this experiment is given in table 3.6.
Neither the different concentrations nor the different treatments had an impact on the parameters end weight, diet consumed, maximum weight, pupation, mortality, duration of pupation or developmental time. However, a clear effect on the emergence success could be found (figure 3.10).

Table 3.6. Results of tested parameters in feeding test 5.
Arithmetic means and standard errors are shown for the tested parameters end weight, diet consumed, maximum weight, pupation, mortality, duration of pupation, developmental time and emergence success. Ctrl: control; bis: β-bisabolene; 7d: treatment with β-bisabolene for 7 days; p: treatment with β-bisabolene until pupation; N = 20.

	ctrl	bis 1.8 ng/g diet (7d)	bis 1.8 ng/g diet (p)	bis 1.8 µg/g diet (7d)	bis 1.8 µg/g diet (p)
End weight (mg)	125.4 ± 13.9	141.2 ± 9.6	120.2 ± 12.7	106.5 ± 10.9	152.2 ± 8. 9
Diet consumed (g)	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.06
Maximum weight (mg)	793.1± 35.9	911.6 ± 49.5	821.5 ± 34.0	873.1 ± 57.8	875.1 ± 29.1
Pupation (day)	36.5 ± 0.8	36.9 ± 0. 8	38.2 ± 1.0	37.2 ± 0.9	35.7 ± 0.9
Duration (days)	16.9 ± 0. 8	16.3 ± 0.6	14.7 ± 1.1	13.5 ± 2.1	17.0 ± 0.9
Developmental time (days)	52.9 ± 0.2	52.7 ± 0.2	52.7 ± 0.4	53.0 ± 0.3	53.1 ± 0.3
mortality (%)	5.0	0.0	15.0	10.0	10.0
emergence success (%)	79.0	75.0	24.0	50.0	26.0

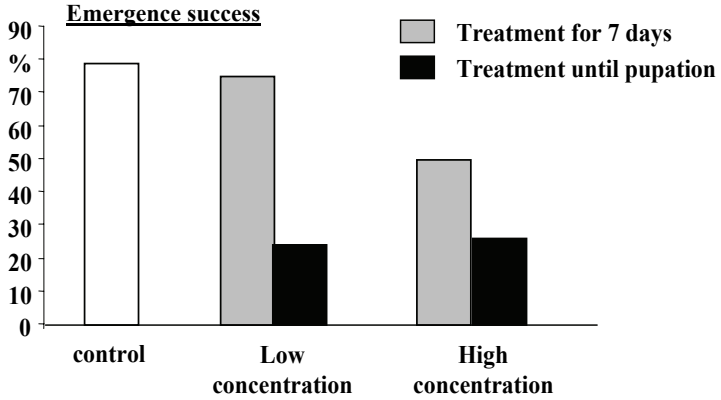

Figure 3.10. Feeding test 5
Arithmetic means are shown for emerging success; N = 20

While the 7-day-treatment with the low concentration showed no effect compared to control with 79 % emergence success, the rate of eclosion was strongly decreased to 24 % in the group which fed on β-bisabolene until pupation. In the group that fed on β-bisabolene for seven days, 50 % of the pupae developed to adults, whereas in the group treated until pupation only 26 % emerged successfully.

3.3.6. Feeding test 6

During the field experiments which were described in chapter 2, a drastic change in the volatile profile of the all corn cultivars was observed in the growing seasons of 2002 and 2003. Instead of the normally occurring sesquiterpenes such as α-ylangene, (*E)*-β-caryophyllene or (*E)*-β-farnesene, two novel components - β- and α-selinene - were found to be the major sesquiterpenoid components of the volatile blend. To investigate possible

toxic or antifeedant effects of these terpenes on *S. littoralis* larvae, both compounds were tested in two different concentrations (17 ng/g diet and 18 µg/g diet for β-selinene; 11 ng/g diet and 1.7 µg/g diet for α-selinene). Due to unknown circumstances, no adult emergence was observed in this feeding test and the parameters duration of pupation, developmental time and emergence success could not be estimated. An overview of the results of all examined parameters in this experiment is given in table 3.7. The most remarkable effect could be found for the group which was treated with α-selinene in low concentration (figure 3.11): the end weight was increased and the caterpillars pupated more than one day earlier relative to the control group. In contrast, the caterpillars that fed on β-selinene in low concentration were significantly smaller after the 7-day-treatment than the larvae reared on the diet without terpenes. In comparison to the control, the higher concentration led to a higher maximum weight in α-selinene and β-selinene treated groups (figure 3.11). The parameters diet consumed and mortality were not affected.

Table 3.7. Results of tested parameters in feeding test 6.
Arithmetic means and standard errors are shown for the tested parameters end weight, diet consumed, maximum weight, pupation and mortality.
Ctrl: control; α-sel: α-selinene; β-sel: β-selinene. Stars (t-test) and double crosses (U-test) show significant differences compared to control (*/ $^{\#}$: p<0,05; **/ $^{\#\#}$: p<0,01; ***/ $^{\#\#\#}$: p<0,001); N = 20.

	ctrl	α-selinene 11 ng	β-selinene 17 ng	α-selinene 1.7 µg	β-selinene 18 µg
End weight (mg)	105.3 ± 4.5	146.3*** ± 9.9	83.3*** ± 4.4	107.3 ± 9.6	97.8 ± 4.6
Diet consumed (g)	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Maximum weight (mg)	697.1 ± 17.5	728.6 ± 30.2	735.6 ± 19.4	791.9$^{\#}$ ± 44.2	813.4*** ± 19.6
Pupation (day)	38.1 ± 0.6	35.3$^{\#\#}$ ± 0.3	38.6 ± 0.8	37.4 ± 0.9	36.8 ± 0.6
mortality (%)	10.0	5.0	10.0	10.0	10.0

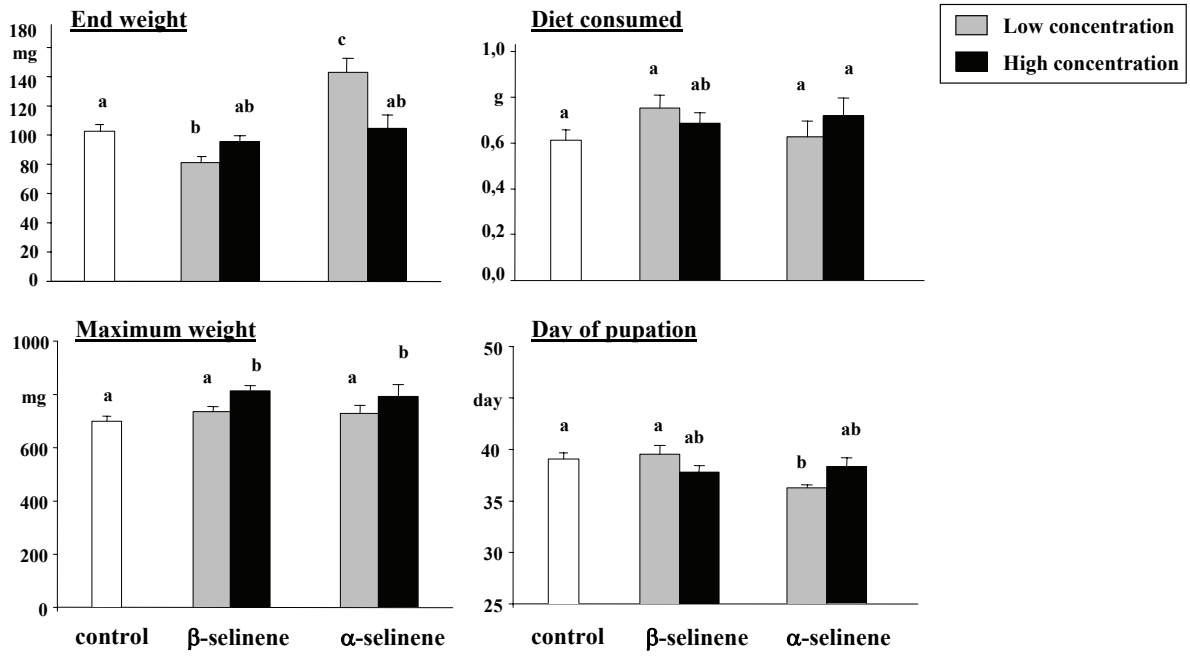

Figure 3.11. Feeding test 6
Arithmetic means and standard errors are shown for parameters end weight, diet consumed, maximum weight and time of pupation. Results are shown for control, β-selinene, and α-selinene. Significant differences compared to control are characterized by different letters; N = 20.

3.4. Discussion

Since terpenes are widely distributed and constitute a part of the natural diet of many herbivores, a lot of studies address the question of how bioactive or toxic these secondary metabolites are to insects and how herbivores can overcome the chemical defenses of their host plants. Terpenes have been reported to be detoxified by a range of cytochrome-P450-dependent monooxygenases, which can be induced by terpene exposure (Pass et al., 2001). According to Foley and Moore (2005), terpenes appear to be low-potency toxins, especially for specialist mammalian herbivores that cause energetically high costs for their detoxification. Hence, herbivores feeding on terpene accumulating or releasing plants should ingest the plant material at a rate that is limited by their ability to detoxify the ingested compounds. However, most of the studies conducted on the bioactivity of terpenoids were performed with terpenoid mixtures from plants that are very rich in aromatic oils and focused on their potential efficiency against pest organisms (e.g. Waliwitya et al., 2005; Koul et al., 2003; Hummelbrunner and Isman, 2001; Jimenez et al., 1996). In the present study, feeding tests were carried out with single terpene compounds starting with concentrations similar to those in corn plants which is in the nanogram range depending on the compound (chapter 2; see also Köllner et al., 2004), and going up to 50 µg/g diet.

Most of the terpenoids showed only little effects on larval development of *S. littoralis*, mainly affecting one fitness parameter or none, as shown for limonene and β-myrcene at the ng-concentrations or for β-myrcene and nerolidol at concentrations of 34 and 39 µg/g diet. The negative impact of terpenes like cycloisosativene at 37 ng/g diet appeared to be compensated prior to pupation, since the end weight was strongly reduced but all other parameters remained unaffected. When *S. littoralis* larvae were treated with terpenes at concentrations ranging from 3-5 µg/g diet, almost all terpenes had positive effects on larval development. In this feeding test, the most pronounced influence was observed in the group which was exposed to nerolidol: while end weight after seven days and diet consumed were strongly increased relative to control, the maximum weight was reduced to about 80 % of the maximum weight of the larvae in the control group. Although the larvae also pupated earlier, the duration of pupation was slightly extended so that the total developmental time was the same compared to control. Interestingly, when the larvae were exposed to terpene concentrations between 18 and 50 µg/g diet, the influences on development were not as pronounced as in the lower concentrations and less parameters were affected. This might be explained by the fact that the gustatory and olfactory chemoreceptors of insects are very sensitive and that compounds which are attractive or neutral at low concentrations may be repellent when they are offered at higher concentrations.

Still, any change even those in one developmental parameter can be crucial for the fitness and survival of herbivores. The development of larvae exposed to (*E*)-β-farnesene at low and high concentrations for instance was retarded compared to the control groups, and thus the pupation was delayed for two and one days, respectively. A 1- or 2-day-delay of pupation and, as a consequence, a delay of emergence can reduce the possibility for finding mates and producing offspring, especially for female *S. littoralis* moths since they mate only about two times throughout their life-cycle while males mate an average of five times (Kehat and Gordon, 1975). A study by Ellis and Steele (1982) examined the fecundity of females of *S. littoralis* in relation to a delay in mating. In this study, the authors could show that both the size of egg mass and percentage of hatching larvae were reduced by a delay in mating. (*E)*-β-farnesene, known to be induced by herbivory, can affect insects in different ways. Mauchamp and Pickett (1987) showed that (*E*)-β-

farnesene and derivatives, when applied to the dorsal surface of larvae of *Dysdera fasciatus*, *Pieris brassicae*, *Manduca sexta* and *Aphis fabae*, had juvenilizing activity interfering with insect development. Although the authors found two species of lepidopteran larvae to be influenced by (*E)*-β-farnesene, this effect could not be confirmed in the present study, maybe due to the different experimental setup. It has been recently shown by Kunert et al. (2005) that exposure of (*E)*-β-farnesene to aphids which use this highly volatile compound as alarm pheromone led to a higher percentage of winged offspring, which are more mobile and leave their host plants. In the present study, when applying the high concentration of (*E*)-β-farnesene to the larvae, they exhibited a significantly lower end weight while consuming almost the same amount of diet compared to the control, suggesting a negative effect of (*E)*-β-farnesene on nutrition or digestion. None of these effects were found in the group which was exposed to (*E)*-β-farnesene at the intermediate concentration. (*E)*-β-caryophyllene, another herbivory-induced terpene, on the other hand, had a positive effect on larval weight and consumption of diet at lower concentrations relative to control, while no effect could be found at the high concentration. Positive effects on larval development were also found for nerolidol, β-ocimene and linalool at low concentrations compared to the control groups. The most dramatic effect was observed for the terpene alcohol linalool: the larvae consumed nearly threefold more diet than the control larvae, were three times heavier after 7 days and pupated almost 5 days earlier relative to control, implying a beneficial effect of linalool. As discussed above, the time point of pupation and, thus, of emergence is important for finding mates and producing offspring. Assuming that female moths rapidly mate and lay their eggs, the early hatching progeny may find more host tissue due to reduced competition for food by other larvae. Furthermore, herbivores that ingest plant material with compounds that act positively on development probably can produce more generations per year since the life cycle is shortened. For a plant, on the other hand, it appears to be detrimental to store compounds which increase the consumption rate of herbivorous insects thus leading to a greater damage on the plant and, moreover, the possibility of suffering more generations of herbivores per year. Given that, the accumulation of linalool might have another reason than the direct defense of the plant against insect herbivores. It has been shown in several studies that linalool can have inhibitory activity on the growth of several fungi (e.g. Nakahara et al., 2003).

It has previously been shown in a study on the Colorado potato beetle *Leptinotarsa decemlineata* that bisabolene has antifeedant effects (Gonzalez-Coloma et al., 1995) and that sesquiterpenes with a bisabolane skeleton can mimic insect juvenile hormones and thus can impair insect development (Bowers et al., 1976). In contrast, none of these effects was observed in the present study, but a clear correlation between duration of exposure to β-bisabolene and the emergence success of the larvae was found. Whereas the emergence success of larvae reared on the terpene-containing diet for seven days was not affected by the low concentration of β-bisabolene and reduced to 50 % by the high concentration compared to control, only 25 % of the larvae emerged successfully when they were exposed to low or high concentration of β-bisabolene until pupation. This indicates that the duration of exposure is crucial, although also the higher concentration during the 7-day-treatment had a effect. Even though β-bisabolene was not lethal for the extremely robust *S. littoralis* and had no influence on weight gain or diet consumed, the accumulation of this terpene in a plant can nevertheless be an efficient chemical defense especially for long-living plants. When herbivores that feed on the plant suffer a reduction in their emergence success later in the season, their offspring may be decreased and the next generation may cause reduced damage to the plant.

During the field seasons 2002 and 2003, a drastic change in the volatile profile of all corn cultivars was observed. Instead of the typical blend of sesquiterpenes, two novel components, β- and α-selinene, were found to be the major sesquiterpenoids. No toxic or deterrent effects of these terpenes on *S. littoralis* were found in the feeding test, but rather beneficial effects, especially for α-selinene offered at the low concentration. Even in the groups that were exposed to high concentrations of β- and α-selinene, a positive influence on larval development was observed, leading to a higher maximum weight relative to control. From previous studies it is known that the composition of stored terpenes plays an important role for the toxicity or deterrence of essential oils since their components can act cumulative or synergistically (e.g. Hummelbrunner and Isman, 2001; Gunasena et al., 1988). For this reason, the results for α-selinene have to be interpreted carefully since the standard consisted of only 60 % pure α-selinene and also included four other sesquiterpenes of ~ 30 % of total. In another study, however, β-selinene was reported to be toxic for *Aedes aegyptii* larvae with 100 % mortality at a concentration of 50 µg/ml and nearly 40 % mortality at 12.5 µg/ml (Momin et al., 2000). This high mortality could not be observed for *Spodoptera littoralis*: in the data presented here the mortality was in the range of 5 to 10 % and did not differ between treatments and control. As discussed in chapter two, herbivory by *Ostrinia nubilalis*, *Spodoptera littoralis* or *Agrotis segetum* did not induce the release of β- or α-selinene. Given these results and the neutral to positive influences on the development found in this bioassay, it is questionable whether β- or α-selinene may have a function as defensive compounds against herbivory by lepidopteran larvae in maize plants.

In many toxicity tests, terpenes were offered to insects on filter paper, applied on the larval surface or even directly injected into the gut lumen. Still, herbivorous insects usually ingest terpenes together with their plant diet and thus feeding tests provide more realistic results using plant material or an artificial diet that mimics the natural food sources. The agarose-diet used in the present study is very simple to prepare and can be easily portioned by pipetting, giving the possibility to observe individual larval development under controlled conditions. Also, the terpenes can be recovered from the diet after one week in a climate chamber. However, the diet became contaminated by microorganisms after four to five days, a problem that could only be eliminated by adding 1 µl formaldehyde per gram diet. This resulted however in a deceleration of larval development and the larvae of *S. littoralis* pupated only after 30 to 40 days while larvae from the same egg clutch reached pupation already after 15 to 20 days when they were reared on plants of *Arabidopsis thaliana* (Meike Burow, personal communication). Consequently, formaldehyde appears to have an impact on larval development and the effects found for the terpenes might have been intensified by the formaldehyde. As illustrated above, terpenes in mixtures can act synergistically, thus it can not be excluded that the observed effects of individual compounds on larval development might have been increased by small quantities of other constituents in the standards (purity of standards between 58 % and 99 %).

In nature, herbivores feeding on plant material are seldomly exposed to single compounds but in most instances ingest plant material containing mixtures of secondary metabolites. The characteristic feature of these mixtures that are induced by herbivore attack or accumulated constitutively in a plant and often are composed of terpenes is their complexity and variability. Furthermore, terpenes have been demonstrated to act synergistically or additively with other compounds as was shown for caryophyllene oxide which synergizes the effect of gossypol on larval development of the lepidopteran species *Heliothis virescens* (Gunasena et al. 1988). Additionally, it has been reported for several moth species that the attractiveness of their host plants is dependent on the volatile

mixture rather than on single compounds thus suggesting that the individual components act synergistically and the mixtures are important olfactory cues for host-plant finding (e.g. Hammack L., 2001; Pivnick et al., 1994; Natale et al., 2003). Hence, it would be interesting to test whether the mixture of terpenes occuring *in planta* might also play a role in direct defense. Köllner et al. (2004) and Schnee et al. (2002) identified and characterized terpene synthase genes which encode the enzymes responsible for the formation of the terpene profile of corn. They defined five distinct groups of sesquiterpene hydrocarbon mixtures whose components always occur in the same relative amounts and in the same plant organs. The corresponding terpene synthase genes were introduced by Schnee et al. (2006) into *Arabidopsis*-plants, which now produce the maize terpenes in amounts similar to corn plants. For this reason, these transgenic lines that constitutively express the maize terpene synthases represent promising tools to examine the role of the terpene mixtures in direct defense against herbivore attack in more detail and should be tested not only with the generalist *S. littoralis*, but also with larvae of more specialized lepidopterans like *Ostrinia nubilalis* and *Agrotis segetum*. In particular, the TPS10-mutant that synthesizes α-bergamotene and (*E*)-β-farnesene, which are known to be induced by *Spodoptera*-infestation on corn plants and recently have been shown to attract parasitic wasps (Schnee et al. 2006), is an very interesting candidate. As illustrated in the present study (*E*)-β-farnesene considerably impaired larval development and might be one of the key compounds that is not only leading the enemies of herbivores to their hosts but furthermore might act directly on the herbivore itself.
Since all feeding tests were conducted with the extremely polyphagous and robust generalist *Spodoptera littoralis*, it is hard to predict whether the compounds that were found to affect *S. littoralis* may also play a role in direct defense against the larvae of more specialized lepidopterans such as *Ostrinia nubilalis* or *Agrotis segetum*. However, even at the low concentrations naturally occurring in corn plants, no lethal but very evident effects on larval development of *S. littoralis* were found. Cycloisosativene, a minor component of the herbivory induced terpene blend and a constituent of the volatile composition of old plants, strongly decreased the end weight after seven days. The same effect was found for δ-cadinene, a sesquiterpene that is present in low amounts in the volatile blend of old corn plants. Also, β-bisabolene was found to impair larval development (table 3.6 and figure 3.10). Therefore, in addition to (*E*)-β-farnesene these sesquiterpenes are potential candidates that might play a role in direct defense of corn plants against a wider variety of herbivores.

4. The impact of abiotic and chemical stress on the volatile emission of corn plants

4.1. Introduction

While the effects of biotic factors like pathogen or herbivore attack on odor induction are well documented, less information is available on how abiotic factors may influence the volatile emission of plants. During their growing season, plants are often exposed to suboptimal conditions such as high or low light, high ozone concentrations, drought or waterlogging stress, and high or low temperature, often in combination with biotic stress. Changes in environmental conditions influence the physiological state of a plant and consequently also the availability of resources to produce volatiles. Takabayashi et al. (1994) found that uninfested leaves of lima bean (*Phaseolus lunatus*) were more attractive to predatory mites after exposition to high light conditions than leaves placed under low light conditions. Recently, Vuorinen et al. (2004) reported that predatory mites were attracted by spider-mite infested as well as by uninfested plants when both were exposed to ozone and could even differentiate between infested and non-infested plants.
In this study, different stress factors were applied to corn plants. These factors will now be introduced in the following paragraph, which discusses briefly physiological changes connected to each stress and if known, the influence on volatile emission of plants.

4.1.1. Temperature stress

According to Schulze et al. (2002) air temperatures between 15-25 °C at day and 10 °C lower temperatures at night are optimal for growth and development of the most plants. When the temperature is above this optimal range, plants undergo a number of morphological changes to avoid overheating such as changes in the orientation of the leaves as short-term acclimations or the development of smaller or hairy leaves as long-term adaptations. Moreover the stomata can be further opened to cool the leaf surface by transpiration or the stomatal density can be increased. Since high temperature and drought are often linked, the stomata may have to be closed to reduce water loss and the temperature of the leaf surface then increases up to 5 °C higher compared to the surrounding air. This might result in a reduction or inhibition of photosynthesis, thus leading to a reduction of the carbohydrate resources. Additionally the metabolic rate is increased, the chemical composition of biomembranes changes to a higher content of saturated fatty acids to ensure the viscosity and stability of the membranes and the metabolic pool can be altered. Often the production of biomass is reduced or inhibited and the plants show abnormal longitudinal growth. C_4-plants, such as maize can photosynthesize more efficiently under conditions of high temperature than C_3-plants, since the opening of the stomata can be minimized to reduce water loss without a loss of CO_2 fixation. Moreover, the optimal temperature for the photosynthesis rate in C_4-plants is in the range of 30-40°C while the optimum for many C_3-plants is between 20-30°C (Larcher, 1994). Independent of anatomical and physiological adaptations, temperatures above a certain range are lethal as essential processes become disturbed and proteins degraded. According to Taiz and Zeiger (2000) temperatures between 49°C and 51°C are lethal for corn plants after ten minutes of exposure. The discovery that terpene synthesis dramatically increases at high temperatures and fumigation with some monoterpenes increases the thermotolerance of the monoterpene-emitting oak *Quercus ilex* (Loreto et al., 1998) led to the hypothesis that the plants might be protected from high temperatures

by terpene emission (Singsaas, 2000). A study on corn seedlings showed that temperatures between 22 °C and 27 °C seemed to be optimal for a higher emission of herbivory-induced volatiles compared to lower or higher temperatures (Gouinguene and Turlings, 2002). Yatagai et al. (1995) reported a higher leaf oil content and terpene emission for *Chamaecyparis obtusa* during summer than in winter. The temperature dependence of terpene release varies however between species. Llusià and Peñuelas (1998) showed that the terpene emission of terpene-storing species like *Pinus halepensis* was more closely related to temperature than in non-storing species where it was rather correlated to photosynthesis rate.

4.1.2. Water stress (Drought/ water deficiency)

Plants under water deficiency develop various morphological adaptations such as a pronounced growth of the root system with more secondary roots, or hairy or waxy leaf surfaces to reduce evaporation. A physiological adaptation to drought is the Crassulaceae acid metabolism which allows CAM-plants to close the stomata during the day and open them during night. An important regulatory hormone for plants under drought stress is abscisic acid (ABA) which is a derivative of zeaxanthin and drives the movements of the stomata. ABA also activates a cascade of transcription factors and promoters to switch on genes that code for protective proteins like aquaporins, dehydrins or ROS-detoxifying enzymes thus leading to a higher stress tolerance, and the stabilization and protection of biomembranes and protein complexes (Schulze et al., 2002). Gouinguene and Turlings (2002) analyzed corn seedlings exposed to soil humidity ranging from 30-80 % and found that the highest amount of volatiles was emitted by plants that were grown on soil with a low humidity. In Mediterranean woody species, on the other hand, the terpene emission rates severely decreases under drought conditions (Llusià and Peñuelas, 1998).

4.1.3. Waterlogging stress (hypoxia)

The term hypoxia is defined as oxygen deficiency due to a full saturation of the soil with water. Under these conditions the oxygen is dissolved in the water instead of being present as a gas in the pores of the soil. Since the gas exchange in fully saturated soils is very slow, the oxygen becomes the limiting factor for growth and development of plants. When the oxygen content in the pores of the soil drops below the critical values of five to ten percent the metabolism is impaired and can be switched from aerobic to anaerobic metabolism for a short time. If oxygen deficiency is present for a longer time the roots can develop anatomical and morphological adaptations such as the development of aerenchyma and an intense internodal growth. After ventilation of plant organs adapted to hypoxia they can be damaged by reactive oxygen species (ROS) since the ROS-detoxifying systems are degenerated (Schulze et al., 2002). In corn plants, the shoot biomass and the leaf area were reduced when they were exposed to waterlogging stress (Lizaso et al., 2001). The youngest adventitious roots were elongated and developed aerenchyma, while the older roots were damaged extensively by the flooding. Another study on flooded corn plants showed heavy stress symptoms such as the production of superoxide and hydrogen peroxide in the leaves and an increase in chlorophyll breakdown and lipid peroxidation (Yan et al., 1996). Little is known about the effects of waterlogging stress on volatile emission. Gouinguene and Turlings (2002) showed a decrease in volatile emission if corn seedlings were exposed to a 80 %-saturated soil. However, these differences were only detected in plants that were mechanically damaged and treated with *S. littoralis* regurgitant and not in undamaged plants.

4.1.4. Ozone stress

Ozone is present in the stratosphere and protects the earth from UV-radiation, while it is continuously synthesized in the lower troposphere due to a reaction of oxygen with nitrogen oxides (NO_x) or, in the presence of sunlight, a reaction with volatiles derived from plants or from fossil fuel consumption. Ozone penetrates the leaf through the stomata and is rapidly degraded in the outer cell layers such that only traces will reach the mesophyll. It can generate reactive oxygen species such as H_2O_2, O^-_2 or •OH which can denature proteins, destroy membrane lipids or enhance the mutation rate via the destruction of nucleic acids, but may also serve as a signal molecule activating defensive genes (Taiz and Zeiger, 2000). Ozone stress and drought stress are often linked since O_3 deactivates the K-/Ca-transporters in the guard cells and the stomatal conductance is decreased. Since O_3 is a polar compound, it hardly permeates through membranes but damages membrane bound components such as SH-containing proteins or structural elements of the cells exposed to oxygen. When occurring at higher concentrations, ozone can destroy pigments in plastidial membranes. A study on *Phaseolus vulgaris* showed that only cells close to the stomata were damaged by photoinactivation of photosystem II (Leipner et al., 2001). Ozone induces plant reactions similar to those elicited by viral or microbial pathogens (Sandermann, 1996). Additionally, it stimulates the production of ethylene and salicylic acid, which operate in different signal transduction pathways and induce changes in gene expression and metabolic processes (Bray et al, 2000). A defensive mechanism of plants under ozone stress is the activation of secondary metabolism such as the synthesis of phenylpropanoids or flavonoids as radical scavengers. Even if plants do not show any damage or stress symptoms after exposition to O_3, they can be predisposed to subsequent abiotic stresses such as water deficiency or high UV-light or to biotic stress like pathogen attack. This predisposition with ozone seems to enhance either the tolerance or the susceptibility of the stressed plant for a second stressor (Sandermann, 1996). According to Schulze et al. (2002) maize plants are relatively sensitive organisms, which can be damaged above an ozone concentration of 0.2 ppm. Several studies were conducted on the effect of ambient or enhanced ozone concentrations on the volatile emission of plants since it is known that volatile organic compounds (VOC) are precursors of ozone. Peñuelas et al. (1998) reported on species-specific effects of ozone exposure: while *Solanum lycopersicum* plants treated with an ozone concentration of 40 nl/l released elevated amounts of VOCs, there was no such effect on *Pinus halepensis*. This species-specific VOC emission in response to ozone was also found in another study with several Mediterranean woody plant species where the authors additionally tested the seasonal changes of volatiles released (Llusià et al., 2002). Nonetheless, some other plant species showed no effects of ozone exposure on volatile release. For instance, Norway spruce plants (*Picea abies*) exposed to ozone concentrations of 40-50 ppb showed compared to their control no differences in volatile emission (Lindskog and Potter, 1994).

4.1.5. Chemical stress (Bromoxinyl)

Herbicides interfere with essential metabolic processes in the plant and either inhibit enzymes or reaction complexes, or induce the formation of radicals thus leading to cell damage. The detoxification of these compounds follows four steps: first the introduction of a hydrophilic group by oxidases or monooxygenases; secondly the conjugation with sugars, mainly glucose, or glutathion by glycosyltranferases or glutathion-S-tranferases, respectively; thirdly the sesquestration of the conjugates in the vacuole or the apoplast via

glutathion pumps (GS-X-pumps) and fourth the immobilization due to modifications in the vacuole or covalent binding in the cell wall (Schulze et al., 2002). Bromoxinyl is a nitrile herbicide that is applied to weeds by spraying on the foliage and only acts on the area where it is applied (Hock et al., 1995). Low doses of this herbicide mimic photosynthetic inhibitors and act on photosystem II (PS II) by binding to the protein D1 (subclass C_3) in the PS II reaction centre, thus blocking electron-transfer and transfer of light energy (Rutherford and Krieger-Liszkay; 2001). The authors hypothesized that the plant is killed by light-induced oxidative stress due to the formation of singlet oxygen in the reaction centre. High doses of bromoxinyl, however, act more like cell membrane disruptors. The half life of bromoxinyl in soil is seven days. Since grass plants seem to be more tolerant to these kinds of non-systemic photosynthesis inhibitors, bromoxinyl is often used in corn cultivation (Kansas State University, Agricultural Experiment Station and Ministry of Agriculture and Food, Canada/ Ontario). Only little information is available on whether exposure to chemicals affects the emission of volatiles in plants. Vercammen et al. (2001) demonstrated the release of several isothiocyanate compounds by *Arabidopsis thaliana* plants upon spraying with Paraquat. An overview over herbicides used in the field site near Halle in the years 2001 to 2003 is given in table 2.2 in chapter 2.

4.1.6. Scope of this chapter

During the field seasons 2002 and 2003, the corn plants were exposed to abiotic factors that changed within the growing season as well as between the years. In 2002, a dry and warm summer was followed by a short period of hard rain and flooding, and the corn plants emitted from beginning of August until the end of the growing season a different volatile blend than the year before (see chapter 2). Unlike 2001, the sesquiterpenes such as β-caryophyllene, α-bergamotene, and β-farnesene could not be detected, and instead two novel sesquiterpenes, β- and α-selinene, were found. These two terpenes were not emitted during the field season 2001 nor by plants under laboratory conditions. This change in the volatile profile of mature corn plants was found among all corn cultivars as well as in all field sites examined. The same phenomenon occurred in August 2003 but in contrast to 2002, the plants were exposed to extraordinary high temperatures and drought for a longer period of time. In 2002 and 2003 but not in 2001, the corn plants were in all field sites infested by larvae of the pest insect *Ostrinia nubilalis* (Hübner). Since the volatile emission of corn plants in the laboratory damaged by larvae of *O. nubilalis* contained neither β- nor α-selinene (see chapter 2) several abiotic factors were chosen to test whether they could have been responsible for the release of β- and α-selinene in the field. To mimic the conditions the corn plants were exposed to in 2002 and 2003 in the field, the abiotic factors high temperature, waterlogging stress, water deficiency and ozone were tested in the same range as they were found in both years in the field. Bromoxinyl was selected since in May 2002 and 2003 but not in 2001 two herbicides were used that interfere with the photosystem II in a similar way as Bromoxinyl (table 2.2 in chapter 2).

4.2. Materials and Methods

4.2.1. Plant cultivation

The maize cultivar "Prelude" was grown in individual plastic pots with 16 cm diameter in clay substrate potting soil (Lasmann, Gross-Hesepe, Germany) with Osmotic fertilizer (Scotts, Nordhorn, Germany) in a climate chamber (York Int., York, USA). The growing conditions were as follows: 22 °C day/ 18 °C night, 65 % relative humidity, 1 mmol/m²/s of photosynthetic active radiation, and with 16 h photoperiod. When the plants were five to seven weeks old, they were repotted into 30 cm diameter plastic pots and moved to the greenhouse where they were grown until maturity.

4.2.2. Plant treatments

Five different experiments were conducted to test the effect of abiotic and chemical stress on the volatile profile of corn. An overview of all experimental conditions is given in table 4.1.

Table 4.1. Conditions during the stress experiments
Σ: sum of all hourly mean O_3 concentrations

Treatment	Plant age at the beginning	Duration of treatment	factor/ concentration	herbivory	volatile collection
High temperature	3 weeks	7 days/ 24 hours	28°C night/ 35°C day	yes/ overnight	10 am - 6 pm
Water deficiency	3 weeks	7 days/ 24 hours	no water	yes/ overnight	10 am - 6 pm
Water-logging	3 weeks	7 days/ 24 hours	constant water	yes/ overnight	10 am - 6 pm
Ozone	mature	**1.** 7 days/ 24 hours **2.** 5 days/ 10am-6pm	**1.** 50µg/m³/h (Σ: 8400 µg/m³) **2.** 260µg/m³/h (Σ: 10400 µg/m³)	no	10 am - 6 pm one day after treatment
Bromoxinyl	**1.** 3 weeks **2.** 8 weeks **3.** mature	7 days/ once daily	**1.** 150 µM **2.** 150 µM **3.** 150 & 300 µM	no	10 am - 6 pm one day after treatment

4.2.2.1. High temperature, water deficiency (drought) and waterlogging stress (flooding)

The plants were grown for three weeks under the conditions described in the upper paragraph (4.2.1.) until the start of the experiment. Per experiment, six plants were treated for seven days and compared to six untreated control plants of the same age.
To test whether elevated temperature had an influence on volatile emission of corn plants, the day temperature was set to 35°C and night temperature to 28°C. To test the effect of waterlogging on corn plants, the plants were allowed to stand in pots with water that was renewed every day and the plants were not allowed to run dry. For the drought experiment the plants were not given any water at all. In each treatment, only the corresponding abiotic factor was changed. Additionally, herbivory treatments were conducted on 4-week-old plants that either had been grown under high temperature, waterlogging stress,

or water deficiency, or on untreated plants. In these experiments, four to five third instar larvae of *Spodoptera littoralis* were placed on a plant that was enclosed in a cage made of Petri dishes and gauze (Röse et al., 1996), and left to feed on the plant overnight.
Since only two volatile collection measurements could be conducted at the same time, parallel volatile collections of one control plant and one treated plant were carried out. To ensure that each plant experienced a seven day treatment before measurement, the exposure of some plants was delayed. The plants were placed in the volatile collection chamber immediately before volatile measurements.

4.2.2.2. Ozone

Ozone for plant treatments was produced by an ozone generator (Sorbios; Berlin, Germany) that was kindly provided by J-P Schnitzler (IMK-IFU; Garmisch-Partenkirchen, Germany). The generator was fed with 1 ml/min oxygen with a purity of 99.8 % (AL; Düsseldorf, Germany) and provided a 1 % ozone concentration. The ozone was pumped into an Erlenmeyer flask and mixed with air to dilute the ozone concentration. This ozone-air mixture was pumped in a treatment chamber of approximately 3 m³ (figure 4.1). The flow rate of the pumps was set to achieve a final ozone concentration of 50 µg/m³/h or 260 µg/m³/h in the treatment chamber.
The treatment chambers were constructed of a wooden frame, covered with a heat resistant foil which allowed the photosynthetically active light to pass, and located in a York climate chamber. The treatment chambers were sealed with adhesive tape once the 3-month-old plants had been placed insede. The ozone-air mixture was dried with a cooling trap to reduce the high humidity inside the chamber before being pumped in. On the outlet of the chamber, the ozone was neutralized by flowing through a copper helix. The plants were exposed to ozone either for eight hours on five consecutive days with a concentration of 260 µg/m³/h or for 24 hours on seven consecutive days with a concentration of 50 µg/m³/h. Three mature control plants were situated in a similar chamber and were treated parallel to the ozone exposition with dried, filtered air over the same period of time (figure 4.1). During the five-day-treatment, the chambers were opened overnight and air was blown into it with a fan.

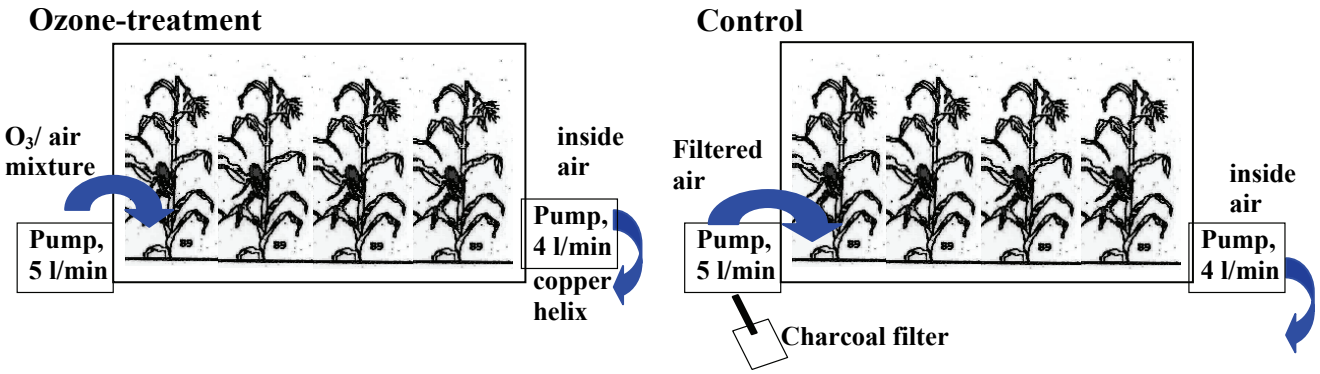

Figure 4.1. Schematic illustration of ozone and control treatment

4.2.2.3. Bromoxinyl

In this experiment, 3-week-old, 8-week-old and at least 3-months-old (mature) plants were treated with Bromoxinyl (PESTANAL; Riedel-de Haën,) for seven days. First, 300 mM and 150 mM stock-solutions were prepared for which 83.1 mg and 41.55 mg Bromoxinyl were dissolved in 1 ml ethanol, respectively. Each stock solution was added

to 1000 ml double distilled water, containing 0.01 % TWEEN20. The control solution consisted of 1000 ml double distilled water, 1 ml ethanol and 0.01 % TWEEN20. Both solutions were applied to the plants for one week by spraying daily at the same time with a household water sprayer until their leaf surface was thoroughly wetted.

4.2.3. Collection and analysis of the volatile blend

The volatile blend of the 4-week-old plants previously exposed to high temperature, waterlogging stress, water deficiency or 150µM Bromoxinyl was collected for eight hours in an automated collection system; the terpene mixture of all mature plants was collected with the field equipment, allowing six parallel measurements. The organ specific distribution of volatiles in mature plants exposed to Bromoxinyl was analyzed with the field equipment using commercially available plastic bags made of inert foil. Both collection systems have been described in detail in chapter 2. The plant volatiles were trapped with SuperQ traps (Alltech). The traps were washed with 200µl CH_2Cl_2 containing 40 ng/µl nonyl acetate as internal standard and the eluted compounds were analyzed by GC-MS (see chapter two).

4.2.3. Statistical analysis

Within the experiments on high temperature, waterlogging stress and water deficiency, One Way ANOVA and Tukey's Post Hoc Tests were used to compare the total emission of volatiles as well as amounts of the individual compounds between all treatments and between the treatments and their corresponding controls. When the assumption of normal distribution within the different groups was not met, Kruskal-Wallis ANOVA on Ranks and Dunn's Post Hoc Tests were performed. For the experiment testing the effect of two different Bromoxinyl-concentrations on mature corn plants, the same tests were used. For both ozone-experiments and the Bromoxinyl-spraying of 3-week-old and 8-week-old plants a t-test was used to examine the total amount of emitted volatiles and the amount of each terpene released from treated plants relative to control. When the required normal distribution or the variance homogeneity of the different groups was not accomplished, a U-test was performed.
Statistical analysis was performed with the program SigmaStat 2.03 (SPSS Inc.) and graphs were created with the program SigmaPlot 7.0 (SPSS Inc.), showing arithmetic means and standard errors if not indicated otherwise.

4.3. Results

4.3.1. Treatment with high temperature

In this experiment 3-week-old corn plants were treated for one week with high temperature. An overview of the results is given in figure 4.1 and table 4.2.
The volatile blend included the monoterpenes (*E*)-β-ocimene and linalool, the homoterpene DMNT and the sesquiterpenes α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, γ-cadinene, and δ-cadinene. The sesquiterpenes (*E*)-β-caryophyllene, (*E*)-α-bergamotene, and (*E*)-β-farnesene are known to be induced by herbivory and accordingly could only be detected after herbivory treatments, whereas the other volatiles were found in all plants (table 4.2). Although there

were qualitative changes in the volatile blend due to the treatment with high temperature found, in this experiment neither β- nor α-selinene could be detected.

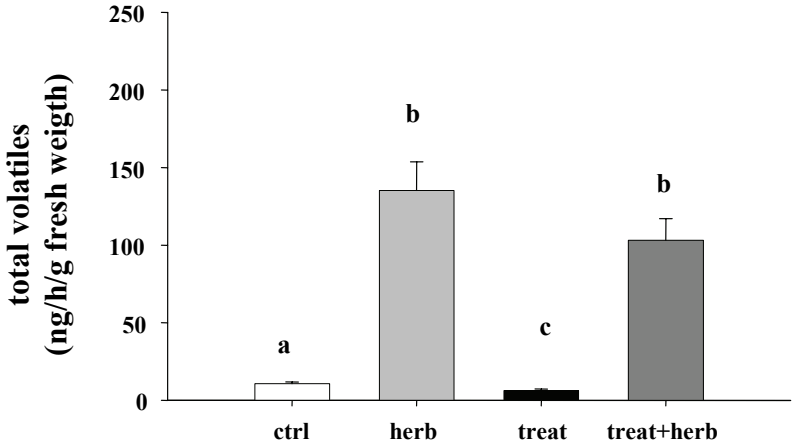

Figure 4.1. Total amount of emitted volatiles after one week of temperature stress
Arithmetic means and standard errors are shown and significant differences between the different groups are characterized by different letters (Tukey's Post Hoc test). ctrl: control; herb: herbivory; treat: treatment; treat+herb: treatment+herbivory; N = 6

Table 4.2. Volatile emission of 4-week-old plants after one week of temperature stress
Arithmetic means and standard errors are shown for the total sum of emitted volatiles, and the homo-, mono- and sesquiterpenes in the control and the treatments temperature, control+herbivory and temperature+herbivory (ng/h/g fresh weight). Stars (One Way ANOVA/ Tukey's Post Hoc Test) and double crosses (ANOVA on ranks/ Dunn's Post Hoc Test) show significant differences compared to the control (*/ $^{\#}$: p<0,05; **/ $^{\#\#}$: p<0,01; ***/ $^{\#\#\#}$: p<0,001); N = 6.

	control	**temperature**	**control + herbivory**	**temperature + herbivory**
total volatiles	10,68 ± 1,12	6,34 * ± 1,08	135,36 *** ± 8,33	103,21 *** ± 3,87
(*E*)-**β-ocimene**	1,54 ± 0,69	1,84 ± 0,43	7,90 ** ± 1,57	7,34 ** ± 1,01
linalool	4,32 ± 0,91	2,22 ± 0,36	45,54 $^{\#\#}$ ± 5,73	37,30$^{\#\#}$ ± 7,15
DMNT	1,75 ± 0,31	1,25 ± 0,27	35,80 $^{\#\#}$ ± 9,05	38,71 $^{\#\#}$ ± 7,76
α-ylangene	0,01 ± 0,001	0,001 * ± 0,0002	0,02 ** ± 0,003	0,01 * ± 0,001
(*E*)-β-caryophyllene	0,00 ± 0,00	0,00 ± 0,00	1,92 $^{\#\#}$ ± 0,38	0,79 ± 0,25
(*E*)-α-bergamotene	0,00 ± 0,00	0,00 ± 0,00	3,80 $^{\#\#}$ ± 1,32	2,48 $^{\#\#}$ ± 0,36
(*E*)-β-farnesene	0,00 ± 0,00	0,00 ± 0,00	10,64 $^{\#\#}$ ± 4,93	6,48 $^{\#\#}$ ± 1,16
(*E,E*)-α-farnesene	3,03 ± 0,33	1,03 ** ± 0,47	28,99 *** ± 5,62	10,09 ** ± 2,20
γ-cadinene	0,01 ± 0,002	0,002 * ± 0,001	0,05 * ± 0,01	0,00 ± 0,00
δ-cadinene	0,02 ± 0,005	0,00 ± 0,00	0,06 * ± 0,02	0,02 ± 0,01

The total quantity of volatiles showed significant differences between the groups (ANOVA on Ranks: $H = 19.253$; $p < 0.001$). No difference were found between the herbivory induced corn plants and the corn plants which were first exposed to high temperature and than to herbivory, but both treatments resulted in a more than 10-times higher emission of volatiles than the control and heat treatment alone (figure 4.1). The plants treated with high temperature alone showed a 40 % decrease in volatiles relative to the control group and the compounds α-ylangene, (*E,E*)-α-farnesene, and γ-cadinene were

found to be released in significantly lower amounts relative to the control (figure 4.1 and table 4.2). Moreover, there were qualtitative changes found between the treatment and the control such that δ-cadinene could be detected in the control plants but not in the plants exposed to high temperature. Most terpenes, such as (*E*)-β-ocimene, linalool, DMNT, α-bergamotene and (*E*)-β-farnesene were emitted by the plants with up to 20-fold amounts relative to control when they were treated with herbivory, independent of previous exposure to high temperature (table 4.2). The sesquiterpenes β-caryophyllene, (*E,E*)-α-farnesene and δ-cadinene on the other hand, were only released in significantly higher amounts after herbivory treatment, but when the plants were treated with high temperature before herbivory, only the emission of (*E,E*)-α-farnesene was significantly increased relative to control (table 4.2). Also the sesquiterpene α-ylangene was significantly increased 2-fold when the plants experienced herbivory after the temperature stress and 4-fold after herbivory alone in comparison to control (table 4.2).

4.3.2. Treatment with water stress

In this experiment, 3-week-old corn plants were left without water for one week. Since the plants exposed to water deficiency showed strong symptoms of desiccation, the total emitted amounts as well as the amounts of all volatiles are given in ng/h/plant. An overview of the results is given in figure 4.2 and table 4.3. The volatile blend included the green leaf volatile (*Z*)-3-hexen-1-yl acetate, the monoterpenes limonene, (*E*)-β-ocimene, and linalool, the homoterpene DMNT, and the sesquiterpenes α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, and δ-cadinene but neither β- nor α-selinene was found in this experiment. There was a qualitative change in the volatile profile of the drought-exposed plants which only emitted linalool, DMNT and α-ylangene while all other volatiles were detected in the control plants except for (*Z*)-3-hexen-1-yl acetate (table 4.3). In both herbivory treatments, no δ-cadinene could be detected but all other volatiles were present.

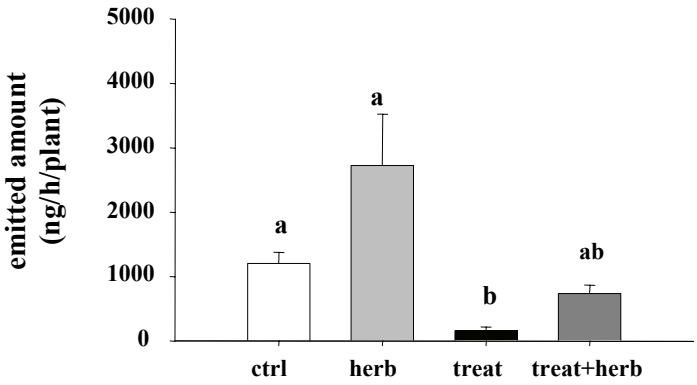

Figure 4.2. Total amount of emitted volatiles after one week of water deficiency stress
Arithmetic means and standard errors are shown and significant differences between the different groups are characterized by different letters (Dunn's Post Hoc test). ctrl: control; herb: herbivory; treat: treatment; treat+herb: treatment+herbivory; N = 6.

In the amount of total volatiles significant differences appeared between the groups (ANOVA on Ranks: $H = 16.289$, $p < 0.001$). However, no significant differences were found between the control plants, the plants that were exposed to both drought stress and herbivory and the group that experienced herbivory alone (figure 4.2 and table 4.3), but when the plants were treated with drought stress, the amount of volatiles was strongly

decreased compared to control plants and herbivore-infested plants (figure 4.2). The control plants and the herbivore-infested plants showed a trend to emit an elevated amount of volatiles compared to the plants exposed to drought or the plants exposed to drought and herbivory, respectively. The most dramatic increase in emission was found in the plants exposed to herbivore-infestation when compared to control: (*Z*)-3-hexen-1-yl acetate was found to be increased more than 300-times in the herbivore-infested plants relative to the control plants, while the release of (*E*)-β-farnesene was increased by the factor 13. DMNT, (*E*)-α-bergamotene and δ-cadinene on the other hand, showed no significant differences in their emission between the control and all treatments (table 4.3). When plants were treated with water deficiency they emitted significantly lower amounts of the terpenes limonene, (*E*)-β-ocimene, linalool, α-ylangene, (*E*)-β-caryophyllene, and (*E,E*)-α-farnesene than control plants. A similar result was found for the sesquiterpene α-ylangene in the plants exposed to herbivory as well as for the monoterpene limonene emitted by plants treated with drought and herbivory.

Table 4.3. Volatile emission of 4-week-old plants after one week of water deficiency
Arithmetic means and standard errors are shown for the total sum of emitted volatiles, the green leaf volatiles and the homo-, mono- and sesquiterpenes in the control and the treatments drought, control+herbivory and drought+herbivory (ng/h/plant). Stars (One Way ANOVA/ Tukey's Post Hoc Test) and double crosses (ANOVA on ranks/ Dunn's Post Hoc Test) show significant differences compared to the control (*/ $^{\#}$: p<0,05; **/ $^{\#\#}$: p<0,01; ***/ $^{\#\#\#}$: p<0,001); N = 6.

	control	**drought**	**control + herbivory**	**drought + herbivory**
total volatiles	1204.16 ± 75.54	158.11 ± 58.96	2722.01 ± 803.60	733.40 ± 131.42
(*Z*)-3-hexen-1-yl acetate	0.00 ± 0.00	0.00 ± 0.00	302.60$^{\#\#}$ ± 55.60	224.81 ± 62.90
limonene	10.29 ± 1.649	0.00$^{\#\#}$ ± 0.00	7.58 ± 2.21	0.00$^{\#\#}$ ± 0.00
(*E*)-β-ocimene	42.25 ± 10.34	0.00$^{\#\#}$ ± 0.00	75.37 ± 25.82	11.58 ± 3.27
linalool	346.69 ± 49.62	113.04$^{\#\#}$ ± 41.21	792.71 ± 308.21	154.60 ± 35.75
DMNT	121.24 ± 11.87	44.92 ± 17.84	200.38 ± 37.22	104.45 ± 31.73
α-ylangene	0.43 ± 0.07	0.15*** ± 0.02	0.16** ± 0.04	0.28 ± 0.03
(*E*)-β-caryophyllene	59.35 ± 21.78	0.00$^{\#\#}$ ± 0.00	8.95 ± 2.74	28.86 ± 11.89
(*E*)-α-bergamotene	17.23 ± 5.56	0.00 ± 0.00	208.57 ± 59.15	32.06 ± 12.79
(*E*)-β-farnesene	47.57 ± 25.93	0.00 ± 0.00	629.30* ± 210.22	46.95 ± 9.81
(*E.E*)-α-farnesene	557.39 ± 140.15	0.00$^{\#\#}$ ± 0.00	496.39 ± 159.40	129.83 ± 45.30
δ-cadinene	1.71 ± 0.48	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

4.3.2. Treatment with waterlogging

During this experiment, 3-week-old corn plants were exposed to soil saturated with water for seven days. The results are shown in figure 4.3 and table 4.4. In the experiment, the following volatiles were found: β-myrcene, hexenylactetate, limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, (*E*)-β-caryophyllene, (*E*)-α-bergamotene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, β-bisabolene, γ-cadinene, δ-cadinene, and β-sesquiphellandrene. While the plants in the control group did not release β-myrcene, (*E*)-α-bergamotene, β-bisabolene, and β-sesquiphellandrene, all volatiles were detected in the treatment groups. Quantitative as well as qualitative changes in the volatile profile were found, but no selinenes were detected in the entire experiment.

In this experiment, significant differences between the groups were found for the total volatile emission (One Way ANOVA: $F = 78.332$; $p < 0.001$). In the plants that experienced both waterlogging and herbivory or herbivory alone, the total amount of volatile emission was increased up to 20 times relative to the control group and up to 10 times relative to the flooding treatment (figure 4.3). Significant differences were also found between both herbivory treatments, with a 30 % higher total emission of volatiles in plants which were treated with herbivores alone (figure 4.3). The only terpenes which showed no differences between the control and the treatment groups were the sesquiterpenes γ- and δ-cadinene and the monoterpene (*E*)-β-ocimene (table 4.4).

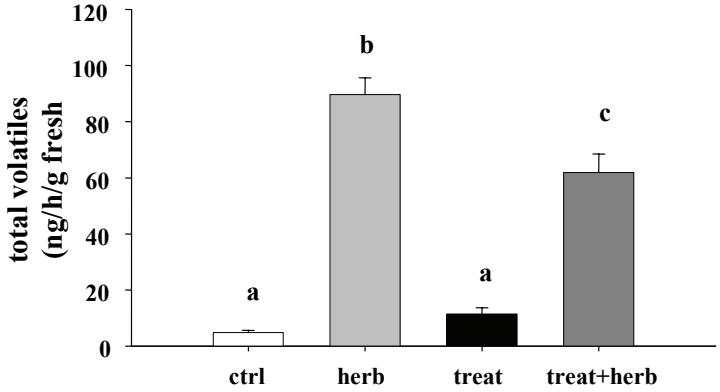

Figure 4.3. Total amount of emitted volatiles after one week of waterlogging stress
Arithmetic means and standard errors are shown and significant differences between the different groups are characterized by different letters (Tukey's Post Hoc test). ctrl: control; herb: herbivory; treat: treatment; treat+herb: treatment+herbivory; N = 6

Table 4.4. Volatile emission of 4-week-old plants after one week of waterlogging stress
Arithmetic means and standard errors are shown for the total sum of emitted volatiles, the green leaf volatiles and the homo-, mono- and sesquiterpenes in the control and the treatments flooding, control+herbivory and flooding+herbivory (ng/h/g fresh weight). Stars (One Way ANOVA/ Tukey's Post Hoc Test) and double crosses (ANOVA on ranks/ Dunn's Post Hoc Test) show significant differences compared to the control (*/ $^{\#}$: $p<0,05$; **/ $^{\#\#}$: $p<0,01$; ***/ $^{\#\#\#}$: $p<0,001$); N = 6.

	control	**flooding**	**control + herbivory**	**flooding + herbivory**
total volatiles	4.85 ± 0.78	11.485 ± 2.229	89.64 *** ± 6.01	61.84 *** ± 6.66
β-myrcene	0.00 ± 0.00	0.008 ## ± 0.002	0.01 ## ± 0.002	0.01 ## ± 0.001
(*Z*)-3-hexen-1-yl acetate	0.49 ± 0.11	0.346 ± 0.070	8.28 ## ± 1.39	6.39 ## ± 2.03
limonene	0.05 ± 0.01	0.087 ± 0.016	0.16 ± 0.03	0.32 *** ± 0.04
(*E*)-β-ocimene	0.37 ± 0.07	0.143 ± 0.028	1.10 ± 0.16	1.59 ± 0.77
linalool	0.68 ± 0.08	1.588 ± 0.435	21.25 *** ± 2.39	11.58 *** ± 1.55
DMNT	0.43 ± 0.08	0.312 ± 0.062	9.92 ## ± 2.17	5.03 ± 1.71
α-ylangene	0.003 ± 0.001	0.007 ± 0.002	0.02 *** ± 0.003	0.004 ± 0.001
(*E*)-β-caryophyllene	0.32 ± 0.08	0.230 ± 0.047	2.30 ### ± 0.32	1.280 ± 0.34
(*E*)-α-bergamotene	0.00 ± 0.00	0.586 ± 0.123	10.31 ## ± 0.94	7.93 ## ± 1.62
(*E*)-β-farnesene	0.21 ± 0.05	1.187 ± 0.340	13.98 ## ± 1.64	9.51 ## ± 1.62
(*E*)-α-farnesene	1.06 ± 0.19	5.121 ± 0.887	12.72 *** ± 1.09	6.456 ** ± 1.62
β-bisabolene	0.00 ± 0.00	0.259 ± 0.157	1.11 # ± 0.28	1.23 ## ± 0.34
γ-cadinene	0.007 ± 0.001	0.012 ± 0.004	0.01 ± 0.004	0.01 ± 0.002
δ-cadinene	0.03 ± 0.004	0.018 ± 0.004	0.01 ± 0.004	0.012 ± 0.005
β-sesquiphellandrene	0.00 ± 0.00	0.811 ± 0.243	5.30 ## ± 1.14	6.25 ## ± 0.77

Beta-myrcene, on the other hand, was emitted in all treatments but not from control plants (table 4.4). Another group of terpenes, including α-ylangene, and (*E*)-β-caryophyllene

showed a 6- to 8-fold emission in the plants that experienced herbivory, but when the plants were exposed to flooding before herbivory, no differences in emission could be shown in comparison to the control (table 4.4). The same was found for DMNT, but here the emission after herbivory was as much as 25-times higher than in the control plants. (*Z*)-3-hexen-1-yl acetate, linalool, (*E*)-α-bergamotene, (*E*)-β-farnesene, and (*E,E*)-α-farnesene were found to be emitted in elevated amounts compared to control by plants that were exposed to flooding and herbivory as well as after exposure to herbivory alone (table 4.4). For these volatiles, the emission was by far more increased in the plants that experienced herbivory alone than in the plants that were exposed to waterlogging stress before herbivory. Plants that were either treated with flooding and herbivory or with herbivory alone emitted β-sesquiphellandrene in similar amounts but this compound was not found in the control. Beta-bisabolene showed a higher emission relative to control when the plants were treated either with herbivory or with flooding before herbivory but, contrary to the above described volatiles, the emission was highest by plants that were exposed to waterlogging and herbivory. The monoterpene limonene was emitted in higher amounts only by plants that were treated with flooding and herbivory, while all other treatments showed no differences relative to control during this experiment (table 4.4).

4.3.4. Treatment with ozone

The control plants of the 7-day ozone experiment (see table 4.1) emitted limonene, DMNT, α-ylangene, (*E*)-α-bergamotene, and (*E*)-β-farnesene, whereas the plants exposed to 50 µg/m^3/h ozone showed the release of all above mentioned volatiles except for (*E*)-α-bergamotene and (*E*)-β-farnesene (table 4.5). Significant differences were neither found in the total sum of volatiles emitted by the ozone-treated plants nor in the emission of limonene, DMNT or α-ylangene.
During the 5-day experiment, the ozone concentration was increased to 260 µg/m³/h and the volatile profile of plants either exposed to ozone or filtered air included linalool, DMNT and α-ylangene (table 4.5). Significant differences between control and ozone-treatment were only found for β-bisabolene which was not present in the plants exposed to ozone (table 4.5), while the total emission of volatiles as well as the release of linalool, DMNT and α-ylangene were in the same range and showed no differences between the groups. In these experiments no β- or α-selinene could be detected.

Table 4.5. Volatile emission of mature plants after ozone stress for 7 days and 5 days
Arithmetic means and standard errors are shown for the total sum of emitted volatiles and the homo-, mono- and sesquiterpenes in the control and the ozone treatment (ng/h/g fresh weight). Double crosses (U-test) show significant differences compared to control ($^{\#}$:p< 0.05; $^{\#\#}$:p< 0.01; $^{\#\#\#}$:p< 0.001).

	control, 7d (N=12)	**ozone, 7d 50 µg/m³/h (N=12)**	**control, 5d (N=10)**	**ozone, 5d 260 µg/m³/h (N=10)**
total volatiles	1.092 ± 0.203	1.183 ± 0.315	0.845 ± 0.059	0.758 ± 0.083
limonene	0.307 ± 0.065	0.523 ± 0.164	0.000 ± 0.000	0.000 ± 0.000
linalool	0.000 ± 0.000	0.000 ± 0.000	0.571 ± 0.040	0.561 ± 0.057
DMNT	0.434 ± 0.077	0.657 ± 0.152	0.206 ± 0.016	0.198 ± 0.029
α-ylangene	0.002 ± 0.001	0.003 ± 0.001	0.0002 ± 0.0001	0.0002 ± 0.0001
(*E*)-α-bergamotene	0.123 ± 0.020	0.000 $^{\#\#\#}$ ± 0.000	0.000 ± 0.000	0.000 ± 0.000
(*E*)-β-farnesene	0.228 ± 0.080	0.000 $^{\#\#\#}$ ± 0.000	0.000 ± 0.000	0.000 ± 0.000
β-bisabolene	0.000 ± 0.000	0.000 ± 0.000	0.069 ± 0.009	0.000 $^{\#\#\#}$ ± 0.000

4.3.5. Treatment with Bromoxinyl

An overview of the results found in these experiments is given in figures 4.4 and 4.5 and tables 4.6 to 4.8.

The volatile profile of 4-week-old plants (table 4.1, experiment 1) included β-myrcene, (*Z*)-3-hexen-1-yl acetate, limonene, (*E*)-β-ocimene, linalool, DMNT, α-ylangene, (*E*)-α-bergamotene, (*E*)-β-farnesene, (*E,E*)-α-farnesene, γ-cadinene, and δ-cadinene (table 4.6). All volatiles, except for α-ylangene which was not found in the control plants, were emitted by Bromoxinyl-treated plants as well as by the control plants. Here, no selinenes but quantitative changes in the volatile blend of BO-exposed plants could be found. When plants of this age were treated with Bromoxinyl, the release of (*E,E*)-α-farnesene, γ-cadinene, and δ-cadinene was significantly reduced by 40 % relative to the control, whereas the emission of all other volatiles and the total emission showed no differences between control and treatment (table 4.6).

Table 4.6. Volatile emission of 4-week-old plants after one week of Bromoxinyl spraying
Arithmetic means and standard errors are shown for the total sum of emitted volatiles, the green leaf volatiles and the homo-, mono- and sesquiterpenes in the control and the treatment bromoxinyl spraying (150 µM) (ng/h/g fresh weight). Stars (t-test) and double crosses (U-test) show significant differences compared to control (*/ $^{\#}$: p<0,05; **/ $^{\#\#}$: p<0,01; ***/ $^{\#\#\#}$: p<0,001); N = 6

	control	**Bromoxinyl, 150µM**
total volatiles	347.090 ± 12.466	266.405 ± 36.726
β-myrcene	0.096 ± 0.008	0.074 ± 0.009
(*Z*)-3-hexen-1-yl acetate	10.231 ± 2.035	11.411 ± 2.996
limonene	1.854 ± 0.297	1.883 ± 0.471
(*E*)-β-ocimene	9.29 ± 0.844	8.825 ± 2.168
linalool	62.921 ± 6.618	75.094 ± 10.467
DMNT	18.763 ± 2.183	27.528 ± 6.203
α-ylangene	0.000 ± 0.000	0.086 ± 0.013
(*E*)-α-bergamotene	4.833 ± 1.295	4.037 ± 1.136
(*E*)-β-farnesene	15.574 ± 3.499	11.214 ± 2.575
(*E,E*)-α-farnesene	222.802 ± 9.608	125.742 $^{\#\#}$ ± 18.588
γ-cadinene	0.321 ± 0.014	0.230 * ± 0.028
δ-cadinene	0.403 ± 0.014	0.281 * ± 0.036

In 9-week-old plants (table 4.1, experiment 2), the sesquiterpenes β-selinene and α-selinene but also the monoterpene limonene were detected exclusively in plants sprayed with Bromoxinyl (table 4.7). Contrary, (*E*)-α-bergamotene and (*E*)-β-farnesene were only emitted by the control plants. In both the control plants and the plants exposed to Bromoxinyl the volatile blend furthermore included (*E*)-β-ocimene, linalool, DMNT, α-ylangene, γ-cadinene, and δ-cadinene (table 4.7). A strong increase in emission was found for DMNT: plants exposed to Bromoxinyl released a 4-times higher amount of this homoterpene than control plants. Although the total emission of volatiles by plants exposed to Bromoxinyl was twice as high as that emitted by the control plants, this difference was not significant. Also, no significant differences could be found for all other terpenes between control and Bromoxinyl-treatment.

Table 4.7. Volatile emission of 9-week-old plants after one week of Bromoxinyl spraying
Arithmetic means and standard errors are shown for the total sum of emitted volatiles, the green leaf volatiles and the homo-, mono- and sesquiterpenes in the control and the treatment bromoxinyl spraying (150 µM) (ng/h/g fresh weight). Double crosses (U-test) show significant differences compared to control ($^{\#}$: p<0,05; $^{\#\#}$: p<0,01; $^{\#\#\#}$: p<0,001); N = 6

	control	**Bromoxinyl, 150 µM**
total volatiles	7.997 ± 1.313	18.959 ± 5.810
limonene	0.000 ± 0.000	0.373 $^{\#}$ ± 0.109
(*E*)-β-ocimene	0.495 ± 0.146	0.753 ± 0.234
linalool	1.759 ± 0.462	2.649 ± 0.808
DMNT	4.168 ± 0.825	14.984 $^{\#}$ ± 4.715
α-ylangene	0.007 ± 0.001	0.005 ± 0.001
(*E*)-α-bergamotene	0.567 ± 0.157	0.000 $^{\#\#}$ ± 0.000
(*E*)-β-farnesene	0.826 ± 0.382	0.000$^{\#\#\#}$ ± 0.000
β-selinene	0.000 ± 0.000	0.081 $^{\#\#}$ ± 0.024
α-selinene	0.000 ± 0.000	0.079 $^{\#}$ ± 0.029
γ-cadinene	0.028 ± 0.009	0.014 ± 0.005
δ-cadinene	0.039 ± 0.014	0.022 ± 0.007

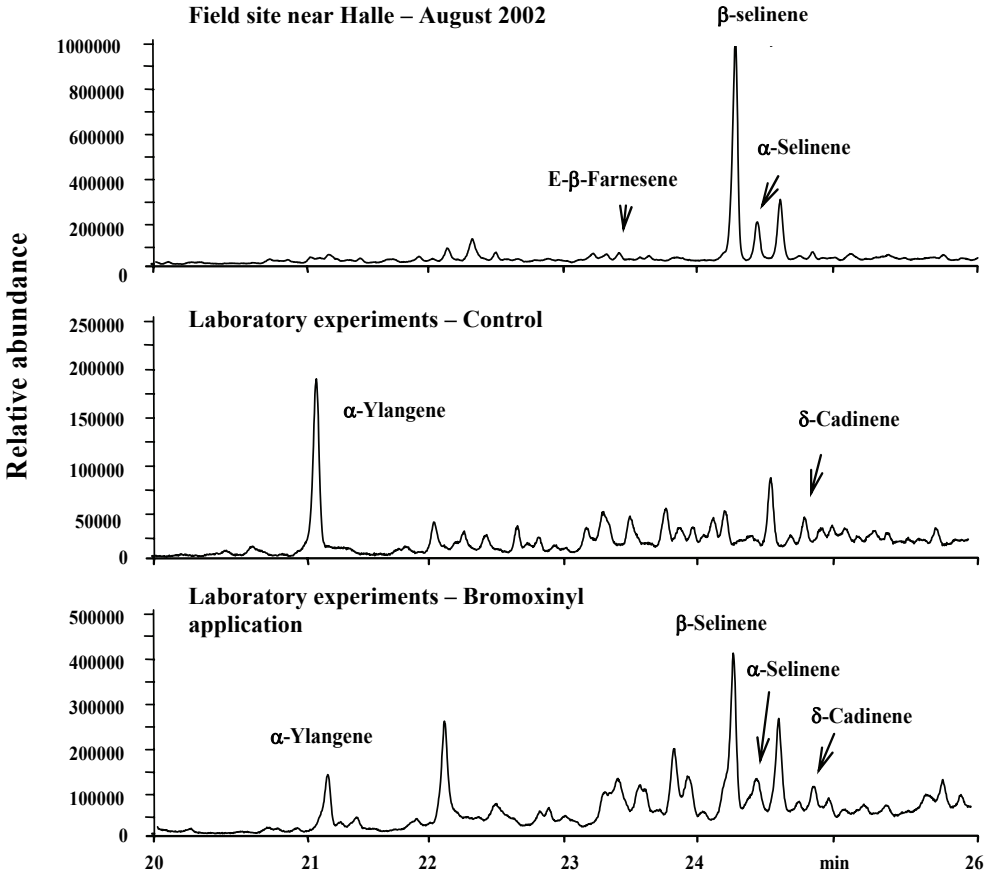

Figure 4.4. GC-chromatograms of the emitted sesquiterpenes of mature corn plants (circa 3-months-old; cultivar Prelude) showing selinene emission in the field and under laboratory conditions.

Also, mature plants (table 4.1, experiment 3) released β-selinene and α-selinene after exposure to Bromoxinyl (table 4.8). Among these two compounds, linalool, DMNT, α-ylangene, and δ-cadinene were detected in the volatile profiles of both treated and control plants. As illustrated in figure 4.4 and table 4.8, β- and α-selinene were exclusively emitted by plants sprayed with BO in both concentrations, thus leading to a volatile emission comparable to the profile found at the end of the field seasons 2002 and 2003.
Significant differences between the group appeared for the total amount of volatiles (ANOVA on Ranks: H = 11.463; p = 0.003). Plants treated with 150 µM Bromoxinyl showed a 8-fold higher total emission of volatiles in comparison to the plants sprayed with 300 µM Bromoxinyl, which was mainly due to a 6- to 8-times higher release of linalool and DMNT by plants exposed to 150 µM Bromoxinyl compared to the 300 µM Bromoxinyl-spraying (table 4.8). However, no significant differences in total emission were found between the control and both Bromoxinyl-treatments (figure 4.5). Although α- and β-selinene were released by both groups of treated plants, their emission was remarkably lower in plants after the application of the higher Bromoxinyl concentration (table 4.8). All other terpenes were emitted in similar or slightly lower amounts compared to control when the plants were exposed to the higher Bromoxinyl concentration.

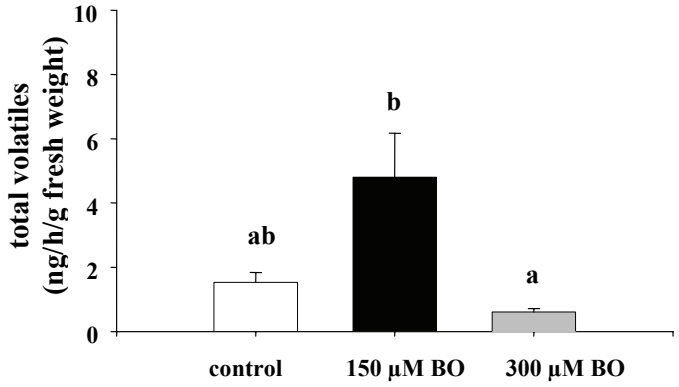

Figure 4.5. Total amount of volatiles emitted of mature plants after one week of Bromoxinyl spraying (150 µM and 300 µM)
Arithmetic means and standard errors are shown and significant differences between the different treatments are characterized by different letters (Dunn's Post Hoc Test); N = 6.

Table 4.8. Volatile emission of mature plants after one week of Bromoxinyl spraying
Arithmetic means and standard errors are shown for the total sum of emitted volatiles and the homo-, mono- and sesquiterpenes in the control and the treatment bromoxinyl spraying at the concentrations 150 µM and 300 µM (ng/h/g fresh weight). Stars (One Way ANOVA/Tukey's Post Hoc Test) and double crosses (ANOVA on ranks/ Dunn's Post Hoc Test) show significant differences compared to control (*/ $^{\#}$: p<0,05; **/ $^{\#\#}$: p<0,01; ***/ $^{\#\#\#}$: p<0,001); N = 6

	control	BO, 150 µM	BO, 300 µM
total volatiles	1.412 ± 0.284	4.799 ± 1.372	0.609 ± 0.108
linalool	0.426 ± 0.060	1.773 ± 0.611	0.227 ± 0.064
DMNT	0.976 ± 0.232	2.642 ± 0.870	0.313 ± 0.064
α-ylangene	0.003 ± 0.001	0.008 ± 0.004	0.002 ± 0.001
β-selinene	0.000 ± 0.000	0.179 $^{\#\#\#}$ ± 0.119	0.038 $^{\#\#\#}$ ± 0.013
α-selinene	0.000 ± 0.000	0.17 ± 0.125	0.022 ± 0.014
δ-cadinene	0.008 ± 0.003	0.028 ± 0.012	0.008 ± 0.004

As shown in figure 4.6, the distribution of sesquiterpenes in mature maize plants differed between the organs examined: inflorescence, youngest leaf, old leaf next to the corn cob (bract) and the leaves around the corn cob (husk). While the sesquiterpene α-ylangene was emitted by all plant organs examined, β-selinene and α-selinene were released only by the husk and the bract. Additionally, the sesquiterpene δ-cadinene was released by the bract. Since the organ specific volatile collections were conducted on two plants only, no quantitative analysis was performed.

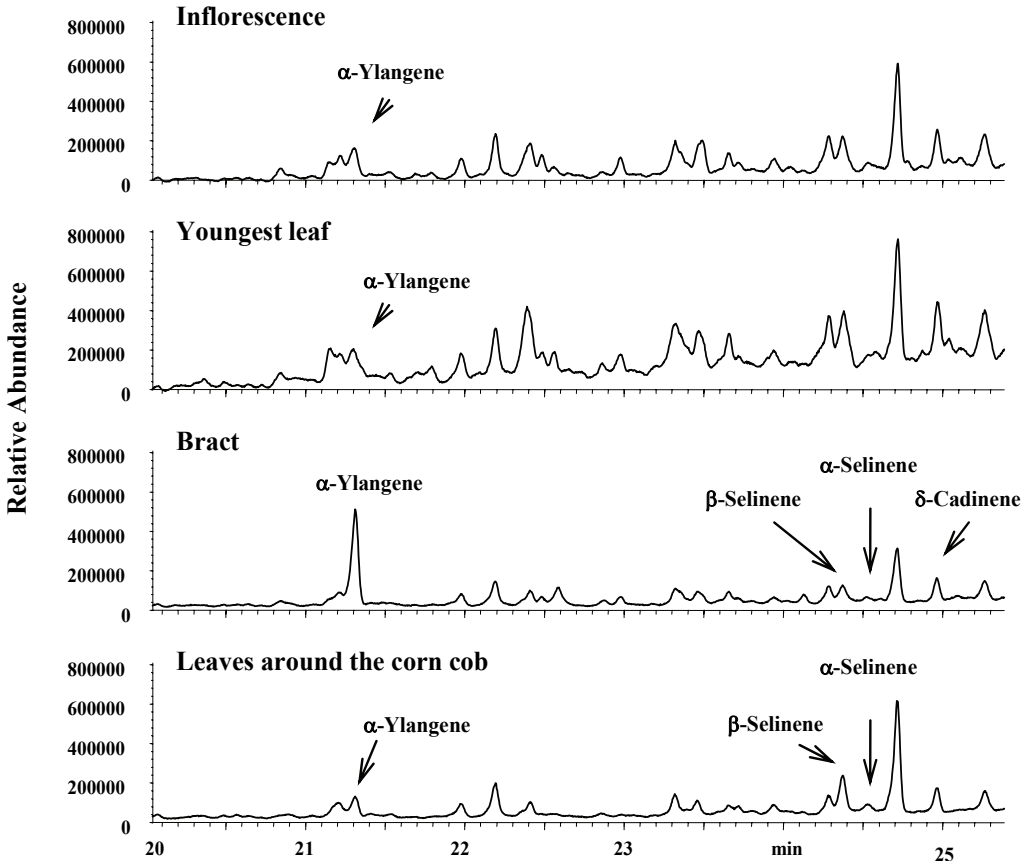

Figure 4.6. Organ specific distribution of the sesquiterpenes in mature corn plants after one week of Bromoxinyl-spraying (150 µM)

4.4. Discussion

In chapter 2 of this thesis, the complex changes in quantity and composition of terpenoid volatiles observed in field grown maize were discussed. Since these changes could not be attributed to differences in herbivore damage or different herbivore species, the present study examined several abiotic factors that maize is subjected to in a field situation and determined whether these factors contributed to the patterns of volatile emission observed in the years 2001 through 2003 in two field sites.

This chapter illustrated that the volatile blends of the corn plants changed qualitatively and quantitatively in all experiments, but the direction and magnitude of these changes were different for each individual terpene and dependent on the type of stress. Most remarkably, the release of β- and α-selinene could be induced by application of Bromoxinyl to corn plants after anthesis.

When plants were exposed to elevated temperatures, the total amount of emitted volatiles as well as the released amounts of most terpenes were decreased relative to the control. This observation contradicts that of a study by Gouinguene and Turlings (2002) on maize seedlings that examined the effect of different temperatures on the emission of corn plants that were either induced with regurgitant from *S. littoralis* or left undamaged. In their study, only the herbivory induced volatile blend was temperature dependent with the highest emissions of volatiles at 22 °C and 27 °C and lower amounts at higher and lower temperatures. In the present study, the uninfested plants, treated with high temperature were exposed to 35 °C and showed a clear decrease in emission relative to the control plants that were cultivated at 22 °C, whereas the induced volatiles were emitted in the same amounts by plants that either experienced herbivory alone or were exposed to elevated temperatures before herbivory.
In the underlying study, the plants that experienced water deficiency alone emitted only linalool, DMNT and α-ylangene. Except for DMNT the emitted amounts were significantly decreased compared to the control. Furthermore, these plants showed a clear tendency to emit a lower total amount of volatiles than the control plants, although these differences were not significant. A similar trend was found for plants that experienced herbivory after drought stress in comparison to plants that were only treated with herbivore-stress. These data again contradict the study by Gouinguene and Turlings (2002) that reported for seedlings on dry soil a higher emission of herbivory-induced volatiles than for plants on soil with a higher humidity. When analyzing volatiles of uninfested plants, the authors found no effect of different soil humidities. They argued that plants under water stress invest more energy in defense compounds after an herbivore attack to protect the vegetative parts because these plants cannot compensate for herbivory by growth. Recently, Vallat et al. (2005) found for the volatile composition of apple-bearing twigs in an orchard that the release of some green leaf volatiles as well as of the monoterpenes camphene and α-pinene was negatively correlated with rainfall thus supporting the hypothesis that plants under drought stress synthesize higher amounts of secondary metabolites. Furthermore, Xin et al. (1997) found higher concentrations of defense compounds in corn plants under stress. In the present study, however, the plants already showed clear symptoms of desiccation and in such a case the production of terpenoid volatiles might be too costly, since terpenoid compounds “are more expensive to manufacture per gram than most other primary and secondary metabolites” (Gershenzon, 1994). According to Schulze et al. (2002) the stomata of plants under drought stress are closed to reduce further waterloss. As the volatile amount emitted by plants stressed with water deficiency was only slightly reduced this may imply that the volatile emission is not dependent on stomatal opening but rather on diffusion through the cuticula as suggested by Köllner et al. (2004).
When waterlogging stress was applied to corn plants, the amount of released volatiles was only increased for β-myrcene but emission remained at the level of the control plants, as far as other volatiles and the sum of volatiles emitted were concerned. Plants treated with both waterlogging stress and herbivory showed a tendency to emit lower amounts than the plants which experienced herbivory alone and were grown on soil with a lower humidity. This corresponds partially to the results of Gouinguene and Turlings (2002) who reported that corn plants exposed to higher soil humidity emitted lower amounts of volatiles than plants treated with lower soil humidity. In their study, this effect could only be detected for plants induced by regurgitant of *S. littoralis* and not for undamaged plants.
Ever since it was discovered that volatile organic compounds are associated with the formation of ozone, many studies were conducted to determine whether ambient or enhanced ozone concentrations influence the volatile emission of plants and how these

volatiles are degraded. Heiden et al. (1999) analyzed the effect of ozone on two tobacco cultivars and Scots pine and found a 40 % increase in release of monoterpenes when the pine was exposed to a long-term treatment with ozone. The two tobacco cultivars also emitted methyl salicylate and several sesquiterpenes, but only the more sensitive cultivar released C_6-volatiles. In the present study, however, no differences between control and ozone-exposed plants could be detected for most volatiles, as well as for the total volatile emission. Neither β-selinene nor α-selinene were detected. Lindskog and Potter (1994) also found no differences in volatile emission between ozone-exposed Norway spruce plants and control trees that were exposed to filtered air. Recently, Vuorinen et al. (2004) reported for lima bean that ozone-exposure and spider-mite infestation induced the release of DMNT, TMTT, and (*Z*)-3-hexen-1-yl acetate whereas the monoterpene (*E*)-β-ocimene was only emitted by the spider-mite infested plants. When testing these odors on predatory mites, the authors found that the mites were equally attracted to unexposed and ozone-exposed plants, but could discriminate between uninfested plants and spider-mite-infested plants when both were previously treated with ozone. This indicates that ozone exposure can initiate the emission of volatiles, but does not interfere with the tritrophic interaction. In the present study the herbivory induced terpenes α-bergamotene and (*E*)-β-farnesene were released by the control plants but not by the plants after ozone-exposure. The tritrophic interaction consisting of maize, lepidopteran larvae and parasitic wasps that was demonstrated by Turlings et al. (1990) could be affected in the present experiments as both (*E*)-α-bergamotene and (*E*)-β-farnesene are important components of the volatile blend to attract parasitic wasps (see Schnee et al., 2006). To determine whether the ozone treatment might interfere with the tritrophic system of corn plants further experiments should be conducted with parasitic wasps such as *Cotesia marginiventris*.

The most interesting result of the experiments presented in this chapter was that the emission of the sesquiterpenes β- and α-selinene could be induced after exposure of 8-week-old or mature plants to Bromoxinyl. In the experiments applying this photosynthesis inhibitor to 3-week-old plants, however, no selinene was detected and neither the total amount emitted nor the amounts of most individual volatiles showed differences between control and treated plants. As demonstrated in table 2.2, only in May 2002 and 2003 herbicides were applied near Halle, which interfere with the Photosystem II whereas pesticides with another mode of action such as root- and shoot-inhibitors or acetolactate synthase inhibitors were applied during all field seasons. The fact that the selinene emission could be induced in the laboratory by exposure of corn plants to a PS-inhibitor corresponds nicely with the selinene emission in the field after herbicide treatment in the years 2002 and 2003 (see chapter 2). Hence, it seems to be likely that both the emission of β- and α-selinene in the laboratory and the release in the field might be a consequence of photo-oxidative stress due to interference in PS II. Moreover, the detection of β- and α-selinene in the late field seasons suggest that only plants after anthesis are able to emit β- and α-selinene. These volatiles were found in the laboratory experiments to be released by the bract and the leaves around the corn cob. According to a study by Köllner et al. (2004) both developmental stages as well as the plant organs differ in the composition of the volatile blend (see also Schnee et al., 2004). To test whether the emission of both selinenes is a result of the blockade of PS II or can also be induced by an inhibition of PS I, further experiments should be conducted with other chemicals that bind to PS II like atrazine and to PS I such as paraquat or rosebengal. Even though the abiotic stress factors high temperature, drought and waterlogging were only applied to 3-week-old plants, it seems doubtful that the emission of β- and α-selinene can also be induced in flowering or mature corn plants by these stress factors since the application of a PS-inhibitor in the field was the only commonality between 2002 and 2003 which was not found in 2001.

Photo-oxidative stress occurs when more light energy is adsorbed than is used by photosynthesis. When binding to PS II, photosynthesis inhibitors such as the herbicide bromoxinyl induce a very specific stress caused by the generation of singlet oxygen in the reaction centre of PS II (Rutherford and Krieger-Liszkay, 2001; Krieger-Liszkay, 2005). Singlet oxygen can either be quenched by β-carotene, lycopene or α-tocopherol, or can react with the D1-protein of the PS II and thereby inhibit forward electron transport. Furthermore, it can activate the up-regulation of genes involved in the molecular defense response to photo-oxidative stress (Krieger-Liszkay, 2005; op den Camp et al., 2003). Although ozone can also interact with the D1-protein and disturb the function of PS II, both selinenes could not be detected in the plants exposed to ozone in this study. Contrary to the specific stress on PS II induced by photoinhibitors, ozone exposure causes a more general stress syndrom, as it not only interferes with PS II but also results in a loss of membrane integrity or damaged membrane lipids, proteins and DNA, leading to effects like reduced yield or premature senescence (Bray et al., 2000; Schulze et al., 2002; Taiz and Zeiger, 2000). Hence, ozone damages the plant cells in various ways, forcing the plant to activate numerous defense mechanisms. Since bromoxinyl and other photosynthesis inhibitors act very specifically, the plant can initiate precise defense mechanisms to compensate this oxidative stress. The emission of β- and α-selinene might serve as such a directed defensive plant response as it was only found in the laboratory when old plants were exposed to bromoxinyl. The release of both selinenes by the bract and the leaves around the corn cob also indicates a defensive mechanism as a plant under stress may have to provide relatively more protection for reproductive organs such as inflorescences and seeds. Mc Key (1974) hypothesized that the chemical protection of seeds is a very high priority as it increases the fitness of the offspring. It should be possible to elucidate the function of selinene by applying it to a corn plant and expose the plant afterwards to different stress factors since it is known how to isolate β- and α-selinene from celery oil (see chapter 3). The impact on the plant could then be determined by subsequent measurement of reactive oxygen species (ROS), radicals or chlorophyll fluorescence (Meyer, 1999; Rusza et al., 1999; Prochazkova et al., 2001; Jiang and Zhang, 2002). Another way to answer the open questions on the function of selinene is the identification of the terpene synthase gene that is responsible for the formation of selinene. After introduction of this gene into *Arabidopsis thaliana*, these transformants should be less sensitive to oxidative stress than control plants that are not able to emit β- and α-selinene.

A number of previous studies, which were also discussed in this chapter, have shown that the composition of a volatile blend is subjected to a wide range of factors such as several abiotic and biotic stresses. Among the known and well-examined factors influencing the regulation of the volatile production other factors may be important that were not recognized up to now. As illustrated in this study the release of selinene was exclusively induced by the exposure of the herbicide bromoxinyl to corn plants after anthesis. This is the first report on a qualitative change of the terpene profile emitted by plants after exposure to herbicides. Thus the choice of a herbicide used in agriculture should be made carefully as it might interfere with the direct or indirect defense of plants by changing the composition of the terpene composition.

5. Final discussion and conclusions

The increasing commercial cultivation of transgenic plants has raised the question whether the introduction of a gene from another organism might also have unintended consequences for the crop or its environment in addition to the desired effects. One of the most intensively discussed examples is Bt-maize, which expresses a gene from the soil bacterium *Bacillus thuringiensis* thus producing a toxin that is designed to kill feeding herbivores. Maize already possesses various natural defenses against herbivores, among which is the release of volatile compounds to attract herbivore enemies as an indirect defense. To examine possible side-effects caused by the introduced Bt-gene on this indirect defense, several transgenic lines derived from different Bt-transformation-events and their corresponding isogenic lines were infested with the lepidopteran *Ostrinia nubilalis* (a target of Bt-toxin) and the non-target lepidopterans *Agrotis segetum* and *Spodoptera littoralis*, and the volatile blend was collected and analyzed for qualitative or quantitative differences of the constituents. Furthermore, the volatile profiles of the same varieties were analyzed over three field seasons in two field sites and the parasitation rates of *S. littoralis* in the field were determined.

Considerable qualitative and quantitative changes were found in the volatile profiles of the maize varieties as a consequence of the transformation with a Bt-coding gene as well as infestation by different herbivores. Furthermore, the lines derived from the different Bt-events MON810 and Bt176 responded differently to infestation by the various herbivores. Whereas infestation of the varieties Valmont (Bt176) and Prelude by the stemborers *A. segetum* and *O. nubilalis* led to the same or even a higher emission of the majority of volatiles in the transgenic line compared to the isogenic line, ranging from two-times for β-sesquiphellandrene to ten-times in case of limonene, the pair of cultivars Navares (Bt176)/Antares showed the opposite tendency after exposure to *O. nubilalis*. However, after damage of these four corn lines by *S. littoralis* the transgenic lines Valmont and Navares released increased amounts of most volatiles relative to their isogenic lines Prelude and Antares. On the other hand, a third pair of cultivars, Novelis (MON810)/ Nobilis, showed quantitative as well as qualitative differences between the lines in both, uninfested or *Spodoptera*-infested plants. After exposure to *S. littoralis*, the transgenic corn line showed an inconsistent induction of individual volatiles out of which five were released in comparable amounts and six in strongly decreased amounts relative to the isogenic line. The only exception was the terpene alcohol linalool, which was considerably increased in the transgenic cultivar.

In the field, significant quantitative differences between all Bt-cultivars and their isogenic lines were found, often depending on the sampling date e.g. a certain year or a certain month. In principle, the differences between transgenic plants and their corresponding isogenic lines were not as pronounced as in the laboratory since only few compounds differed significantly between Bt- and non-Bt-plants. Similar to the laboratory data, the corn lines of the event Bt176 showed no qualitative differences in volatile profile compared to their isogenic lines and significant quantitative changes appeared only for a few volatiles. On the other hand, the volatile emission of the transgenic cultivar Novelis (MON810) differed quantitatively from the isogenic line in the case of eight compounds and also qualitatively in the case of one compound, which was exclusively released by the isogenic line. As illustrated above, the emission of individual volatile compounds by maize plants can be directly influenced by the transformation with a Bt-coding gene. Furthermore, the effect on the volatile emission might also be subjected to a combination of the Bt-tranformation and a certain time of the year depending on the developmental stage of a plant (see Köllner at al., 2004) maybe in combination with abiotic factors or a

certain year with different climatic conditions (e.g., Hern and Dorn, 2003; Vallat et al., 2005; Llusià et al., 2002). Since these environmental effects can interact in numerous ways with the plants, the volatile release in the field by transgenic corn plants is hard to predict (see also Crawley, 1999). However, data from the field and particularily the lab indicated that isogenic and transgenic plants differ considerably in their inducibility by different feeding herbivores, and abiotic factors. The authorization of both Bt-events for commercial cropping should be reconsidered, in particular in case of MON810 since plants derived from this event are impaired in their ability to produce and emit volatile compounds and additionally showed reduced food quality (public available data on http://www.biosicherheit.de/mais/308.doku.html). Also other studies on Bt-corn in the field showed that the introduction of a Bt-coding gene influenced the metabolism considerably (Ma & Subedi; 2005; Catangui and Berg, 2002). Interestingely, in both studies no yield advantage of Bt-lines in comparison to their isogenic cultivar could be shown when the infestation with *O. nubilalis* was low to moderate.

The high variability in volatile composition and total amounts released in response to the damage by various herbivores as well as on the climatic changes during the season raised the question, which function the volatile release by a plant might have in the interactions with other organisms. Terpenes are one of the largest groups of secondary metabolites and have been shown to be involved in interactions of plants and insects either indirectly as volatile compounds, which attract predators or parasitoids of herbivores (e.g. Dicke et al., 1990; Llusià & Peñuelas, 2001; McCall et al., 1994; Mattiacci et al., 1994; Röse et al., 1997; Dicke and Sabelis, 1988; Rasmann et al., 2005) or as feeding deterrents or toxins that act directly on the feeding herbivore (e.g. Tripathi et al., 2003; Mauchamp and Pickett, 1987; Bowers et al., 1976; Waliwitya et al., 2005; Koul et al., 2003; Hummelbrunner and Isman, 2001; Jimenez et al., 1997). Indirect defense has been previously described for maize plants (Turlings et al., 1990), but it is still unclear whether the terpene mixtures in corn plants can also serve as direct defense against herbivores. To examine this open question, twelve individual terpenoid compounds that are synthesized by the corn lines used in this study or described for other corn cultivars (Köllner et al., 2004 and Gouinguenè et al., 2001) were tested for their toxicity against the generalist herbivore *S. littoralis*. The terpenes were added to artificial diets in concentrations as they naturally occur in the corn plants to correlate their effect to a potential function in direct defense. In further experiments, increased concentrations such as those usually found in terpene-rich plants like *Thymus*- or *Gossypium*-species were used to identify those compounds that potentially affect the larval development of lepidopteran herbivores at higher concentrations. Among the tested compounds, none showed lethal effects and most terpenoids examined, such as β- and α-selinene, either acted neutrally or showed beneficial effects on larval development. However, for four compounds strong effects on larval development of *S. littoralis* were found even at the lowest concentrations. (*E*)-β-farnesene, which has been demonstrated to be one of the key compounds in the indirect defense of maize plants (Schnee et al., 2006) significantly prolonged developmental time until pupation for two days and thus acted directly on the herbivore itself. A two-day delay in pupation and consequently, of emergence can reduce the possibility to find mates and produce offspring, since moths mate only up to five times throughout their life cycle (Kehat & Gordon, 1975) and the percentage of hatching larvae can be reduced by a delay in mating (Ellis & Steele, 1982). Cycloisosativene and δ-cadinene were shown to decrease the weight of the larvae significantly, whereas β-bisabolene diminished the emergence success after exposure to this compound until pupation. These compounds could act in direct defense, since decreased larval weight might lead to a reduced fecundity and/ or a longer developmental time. Furthermore, a longer developmental time can increase the

predation risk and decrease the larval reproductive fitness. In nature, herbivores feeding on a plant are rarely exposed to single defensive compounds but in most cases consume plant material containing a variety of secondary metabolites, which are often complex and variable mixtures of terpenes. It has been shown in previous studies that the composition of stored terpenes plays an important role for the deterrency or toxicity of essential oils as their components can act additively or synergistically (Hummelbrunner and Isman, 2001; Gunasena et al., 1988). Supplementary to this characteristic trait of terpene mixtures it was demonstrated on several moth species that the attractiveness of their host plants is dependent on the volatile profile, which have to contain one certain or several individual key compounds. This suggests that these blends are important olfactory cues for host-plant finding (e.g. Hammack L., 2001; Pivnick et al., 1994; Natale et al., 2003). Furthermore, terpenoids have been shown to act indirectly as volatile compounds, which attract predators or parasitoids of herbivores (e.g. Dicke et al., 1990; Llusià & Peñuelas, 2001; Rasmann et al., 2005). Hence, the high variability of terpene composition as demonstrated in the field might have considerable consequences on the interactions with other organisms either directly on a herbivore feeding on the plant or indirectly by influencing the attractiveness of the plant to predators and parasitoids. For instance, cycloisosativene, δ-cadinene, β-bisabolene and (*E*)-β-farnesene, sesquiterpenes that were demonstrated in the underlying study to interfere with larval development of *S. littoralis* and are constituents of the volatile blend of the examined corn lines, were not released by several maize cultivars at the end of the field seasons 2002 and 2003. This might have resulted in a decreased direct defense of the plants and furthermore, the attractiveness of the plant to parasitoids could have been declined due to the lack of (*E*)-β-farnesene (see Schnee et al., 2006), thus interfering additionally with the indirect defense of the maize plants.

Plants in the field are exposed to climatic conditions such as drought, high temperature, or increased ozone concentrations that change over time and thus also might influence the volatile profile as shown in several studies in the laboratory and under field conditions (Gouinguene and Turlings, 2002; Yatagai et al., 1995; Llusià and Peñuelas, 1998; Peñuelas et al., 1998; Llusià et al., 2002). Pesticides have also been discussed to influence the volatile emission of plants (Vercammen et al., 2001; Vallat et al., 2005). It was discovered by Loreto et al. (1998) that terpene synthesis dramatically increases at high temperatures and that fumigation with some monoterpenes increases the thermotolerance of the monoterpene-emitting oak *Quercus ilex*. This led to the hypothesis that plants might be protected from high temperatures by terpene emission (Singsaas, 2000). Furthermore, when applying ozone to plants, terpene release is often increased (e.g. Peñuelas et al., 1998). In the presence of ozone, terpenes are oxidized (e.g. Calogirou et al., 1999) or form aerosols (Jenkin et al., 2000) suggesting that terpenes can have a protective role against various oxidative stresses. For other abiotic factors such as water deficiency or waterlogging (hypoxia) it is not yet known whether changes in volatile composition are unavoidable consequences of the abiotic factors or adaptations of plants for protection. Generally, the individual components differ quantitatively in response to a certain stress thus changing the proportions within the volatile composition. However, a field study also reported a qualitative change in the volatile composition of apple trees in correlation to drought. Here, the monoterpene camphene was additionally released, whereas the composition of the other volatiles remained the same (Vallat et al., 2005). In the present field study, however, a more drastic qualitative change in the volatile profile was observed at the end of the field seasons 2002 and 2003. At that time, the whole sesquiterpene-fraction found in the field in 2001 was not present and the two sesquiterpenoids β-selinene and α-selinene, which were not yet described for commercial

corn cultivars, were emitted. The composition of the mono- and homoterpenes, however, did not change. This is the first report on such a drastic qualitative change of a terpene profile emitted by plants in the field. Interestingly, this complete switch was found in all cultivars and in both field sites indicating that it was not a local phenomenon. As an infestation of the corn plants with larvae of *Ostrinia nubilalis* was observed only in 2002 and 2003, the release of these two compounds might have been induced by feeding of this lepidopteran herbivore and could play a role in the direct defense of the maize plants. However, after exposure of maize plants to *O. nubilalis* in laboratory experiments neither β-selinene nor α-selinene were found. Although Momin et al. (2002) reported β-selinene to be lethal for 40 % of the mosquito larvae at 12 µg/g diet, feeding tests with both selinenes on the generalist *S. littoralis* at comparable concentrations revealed no negative effects on larval development. Hence, it seems unlikely that β- or α-selinene might play a role in the direct defense of corn plants.

In order to elucidate the factors that induced the release of β-selinene and α-selinene in the field, corn plants were exposed to several abiotic factors like high temperature, water deficiency, hypoxia, elevated ozone concentrations, or the application of a herbicide in laboratory experiments. The results of these studies demonstrated that the volatile blends of the corn plants changed qualitatively and quantitatively, but the direction and magnitude of these changes were different for each individual terpene and dependent on the type of stress. When plants were exposed to elevated temperatures, for example, the total amount of emitted volatiles as well as the individual amounts of most terpenes was decreased relative to the control. The plants that experienced water deficiency, a stress that is often correlated with high temperature, emitted only few terpenoid compounds and those in significantly decreased amounts compared to the control. When waterlogging stress was applied to corn plants, the emitted amount was only increased for one compound but remained at the level of the control plants, as far as any other volatiles and the sum of volatiles emitted were concerned. After exposure to ozone, however, no significant quantitative differences between control and ozone-exposed plants could be detected for most volatiles and neither for the total volatile emission. Interestingly, the terpenes (*E*)-α-bergamotene and (*E*)-β-farnesene, which are known to be highly inducible by herbivory, were released by the control plants but not by the ozone-treated plants. This indicates that the terpene synthase gene TPS10, which is responsible for the formation of (*E*)-α-bergamotene and (*E*)-β-farnesene and is important for the attraction of parasitic wasps (Schnee et al., 2006), might have been downregulated by ozone. Recently, a study on lima bean reported that ozone exposure can initiate the emission of volatiles such as DMNT, TMTT, and (*Z*)-3-hexen-1-yl acetate, but does not interfere with the tritrophic interaction (Vuorinen et al., 2004). However, further experiments have to be conducted to clarify the question whether the absence of (*E*)-α-bergamotene and (*E*)-β-farnesene in ozone-exposed maize plants might interfere with the tritrophic interaction.

Although in these experiments dealing with biotic or abiotic stress factors quantitative and/ or qualitative changes in the volatile blend were found, neither β-selinene nor α-selinene could be detected. Most interestingely, their emission was induced after exposure of 8-week-old or mature plants to the herbicide Bromoxinyl. In the experiments applying this photosynthesis inhibitor to 3-week-old plants, however, no selinene was detected. As demonstrated in table 2.2, herbicides that interfere with the Photosystem II were applied to the field site near Halle only in 2002 and 2003 thus indicating that the emission of β- and α-selinene in the laboratory and the release of these terpenes under field conditions might be a consequence of photo-oxidative stress. Photo-oxidative stress occurs when more light energy is adsorbed than can be used by photosynthesis. When binding to PS II, photosynthesis inhibitors such as the herbicide Bromoxinyl induce a very specific stress

caused by the generation of singlet oxygen in the reaction centre of PS II (Rutherford and Krieger-Liszkay, 2001; Krieger-Liszkay, 2005). Unlike ozone-exposure that also can disturb the function of PS II, the stress induced by exposure to this herbicide is a very specific and moreover an artificial interference with the physiology of a plant. It can be speculated that the release of selinene in response to the chemical stress induced by Bromoxinyl might serve in a very specific role against photo-oxidative stress. The detection of β- and α-selinene in the late field seasons suggests that only plants after anthesis are able to emit β- and α-selinene. These volatiles were released in the laboratory experiments by the bract and the leaves around the corn cob. According to a study by Köllner et al. (2004) both developmental stages as well as plant organs differ in the composition of their volatile blends. The release of both selinenes by the bract and the husk also indicates a defensive mechanism as a plant under stress has to protect reproductive organs such as seeds with high efficiency. Mc Key (1974) hypothesized that the chemical protection of seeds is important as it directly increases the fitness of the offspring. The release of both selinenes by corn plants after anthesis, which was found in the laboratory after application of Bromoxinyl, was also found in the field although corn plants were sprayed with PS-inhibitors earlier in the season. Hence, the induction of the selinenes might be a process, which can already be triggered in young plants. Since plants under field conditions are not only exposed to a single stress but to a combination of abiotic and biotic stress factors, the effect induced by the herbicides might have been intensified by the climatic conditions such as described above. Furthermore it is known that several abiotic stress factors can be linked such as high temperature/drought or ozone/drought and that plants after exposure to ozone can be predisposed to subsequent abiotic stresses, for example, water deficiency or high UV-light or to biotic stress like pathogen attack. This predisposition seems to enhance either the tolerance or the susceptibility of the stressed plant for a second stressor (Sandermann, 1996). Further experiments need to be conducted to investigate the regulation of α- and β-selinene emission in more detail and whether a pretreatment with a PS-inhibitor enhances the tolerance or the susceptibility of the plant to subsequent stress factors. After applying PS-inhibitors such as Bromoxinyl or Paraquat to 3- to 4-week-old maize seedlings, the plants should be exposed to different abitotic stress factors like enhanced ozone concentrations, high temperature, drought or hypoxia, and the volatile emission should be analyzed after anthesis.

In general, grass plants seem to be more tolerant to non-systemic photosynthesis inhibitors (Kansas State University, Agricultural Experiment Station and Ministry of Agriculture and Food, Canada/ Ontario) thus indicating that they developed adaptive mechanisms, which might not be present in dicotyledons. The release of the selinenes might be a possible mechanism to protect the maize plants from oxidative stress. Whether this is a specific phenomenon in the maize cultivars examined in the present study, or can also be found as a defensive mechanism in other maize varieties or even other monocotyledons remains to be elucidated by analyzing different maize varieties and monocotyledons. Comparison of the volatile release of monocotyledonous plants with that of dicotyledonous plants after application of photosynthesis-inhibitors could further clarify if this drastic qualitative change in volatile composition is a phenomenon present in both large divisions of plants.

The release of volatile terpenes after insect herbivory is an important plant trait that can limit further herbivore damage by attracting herbivore enemies. This study investigated the influence of a number of factors on volatile terpene emission from maize in the context of their effect on plant defense. Laboratory and field experiments showed that various abiotic stresses and the damage of herbivores, in combination with the introduction of a gene encoding Bt-toxin, caused significant variation in the volatile profile and the total amount of emitted volatiles. The most dramatic change in volatile terpene composition observed in the field was traced not to herbivory or transformation by the Bt-toxin gene, but rather to exposure to an herbicide that inhibits photosynthesis. Treatment with Bromoxinyl led to an emission spectrum dominated by two bicyclic sesquiterpenes, α- and β-selinene, that were not found to be emitted under any other conditions. This is the first report that herbicide treatment causes such a profound change in volatile composition, and suggests that the choice of a herbicide might unintentionally alter the terpene composition of plants and hence interfere with plant defense.

Overall, these findings highlight the myriad of different factors that can alter maize terpenes emission, including modern agricultural practices, such as the use of herbicides or certain transgenic cultivars. Since it has been shown that specific volatiles or volatile blends are important in attracting herbivore enemies for indirect defense, further longer-term studies are warranted to insure that agricultural interventions do not inadvertently compromise endogenous plant defense mechanisms.

6. Summary

The volatile profile of three transgenic maize lines, derived from two different Bt-tranformation events, were analyzed in the greenhouse either after exposure to herbivory by *Spodoptera littoralis*, *Ostrinia nubilalis* or *Agrotis segetum* as well as from uninfested plants and compared to that of the corresponding isogenic corn lines. Thereby, one green leaf volatile, four monoterpenes, one homoterpene and ten sesquiterpenes were identified and a high variability in both quantity and composition of the blend was found depending on the herbivore species feeding on the plants. The emission of Mon810-plants either uninfested or after exposure to the herbivore *Spodoptera littoralis* revealed qualitative and quantitative differences in the volatile composition between the transgenic plants and their isogenic lines. Most remarkable, the two sesquiterpenes α-ylangene and β-sesquiphellandrene were found to be exclusively released by the isogenic corn variety. Moreover, the transgenic corn line emitted lower amounts of most individual volatiles except for one compound, which was significantly increased in the emission relative to the isogenic cultivar. The Bt176-plants, on the other hand, showed only quantitative differences in the volatile release between isogenic and transgenic cultivars for untreated plants and after infestation with *S. littoralis, O. nubilalis* or *A. segetum*. Thereby, the transgenic line released significant enhanced amounts of most individual compounds relative to the isogenic line. When comparing the volatile composition of uninfested plants and of plants after damage by the various herbivores, both qualitative and quantitative differences were found for the total amount as well as in the majority of individual released volatiles. Hence, no consistent pattern appeared with regard to the up- or downregulation of the synthesis of individual volatiles.

The volatile blend of the same cultivars was examined in two field sites over three growing seasons. In principle, the same profile as in the greenhouse was found except for one sesquiterpene that could never be detected in the field. Bt176-plants differed quantitatively in the volatile composition to their isogenic lines, while the MON810-plants again showed qualitative and quantitative differences compared to the isogenic line. However, the composition as well as the amounts emitted showed a high variability in dependence on the sampling date, e.g. a certain month or year. Unlike 2001, where no qualitative change in the volatile profile could be detected over time, the volatile composition at the end of the field seasons 2002 and 2003 differed drastically from that released at the beginning of the season. At that time two novel sesquiterpenes, β- and α-selinene, which were not yet described for commercial corn lines, were released instead of the usually occuring sesquiterpenes. Interestingly, this drastic change was observed in all examined corn lines and in both field sites, indicating not a local phenomenon.

To examine the effect of twelve terpenoid compounds identified in the volatile blend of the studied corn lines and other commercial maize cultivars on larval development and whether they play a role in the direct defense of maize plants, feeding tests were conducted with an artificial diet given to larvae of the generalist herbivore *S. littoralis*. Most terpenoids showed neutral or positive effects, but four interfered very evidently with larval development of *S. littoralis* even at the low concentrations that have been described for corn plants. (*E*)-β-farnesene prolonged developmental time until pupation for two days, whereas cycloisosativene and δ-cadinene decreased the weight of the larvae. Beta-bisabolene, however, diminished the emergence success after exposure of *S. littoralis* to this compound until pupation.

Four different stress factors - high temperature, drought, waterlogging and elevated ozone concentrations - and the herbicide Bromoxinyl were applied to corn plants to examine the reason for the drastic change of the sesquiterpene profile at the end of the field seasons 2002 and 2003. In every experiment, quantitative and qualitative differences in the volatile blend of the treated plants were detected relative to the untreated controls, but only the application of the photosynthesis inhibitor Bromoxinyl to corn plants after anthesis could induce the release of both selinenes. Thereby, the emission showed an organ-specific distribution such that β- and α-selinene were released exclusively by the bract and husk, thus possibly indicating a protective function of both selinenes in response to photo-oxidative stress. This is the first report on a qualitative change of the volatile profile after the exposure of plants to a herbicide.

7. Zusammenfassung

In der vorliegenden Studie wurde das Duftstoffprofil von drei verschiedenen transgenen Maiskultivaren unter kontrollierten Laborbedingungen analysiert und mit dem der entsprechenden isogenen Kontrolllinien verglichen. Innerhalb des Duftstoffprofils konnten ein „green leaf volatile“ (GLV), vier Mono-, ein Homo- sowie zehn Sesquiterpene identifiziert werden, die bereits aus anderen Maissorten bekannt sind. Dabei zeigten ungehandelte und durch Fraß des Herbivoren *Spodoptera littoralis* induzierte Pflanzen des Bt-events MON810 sowohl qualitative als auch quantitative Unterschiede in der Komposition der Duftstoffe im Vergleich zur isogenen Linie. Beispielsweise wurden die zwei Sesquiterpene α-Ylangen und β-Sesquiphellandren ausschließlich von der isogenen Maislinie emittiert. Mit Ausnahme eines Terpenalkohols, der von der transgenen Linie in signifikant erhöhten Mengen abgegeben wurde, war die Emission der meisten Substanzen der transgenen Pflanzen signifikant geringer im Vergleich zur isogenen Linie. Im Gegensatz dazu unterschied sich die Duftstoffabgabe von Pflanzen des Bt-events Bt176 nach Fraß durch *Spodoptera littoralis*, *Ostrinia nubilalis* oder *Agrotis segetum* sowie von unbehandelten Pflanzen des gleichen Transformations-events lediglich quantitativ von der der entsprechenden isogenen Kontrolllinien. Hierbei gaben die transgenen Linien größere Mengen an volatilen Stoffen im Vergleich zu den isogenen Kontrollinien ab. Darüber hinaus zeigte die Duftstoffabgabe aller Maislinien nach Behandlung mit verschiedenen Lepidopteren-Arten eine hohe quantitative und qualitative Variabilität innerhalb der jeweiligen Sorte, wobei sich jedoch kein einheitliches Bild bezüglich der Erhöhung oder Verringerung der einzelnen Komponenten nachweisen ließ.

Um mögliche Nebeneffekte des eingefügten Bt-gens auf die Duftstoffabgabe im Freiland zu untersuchen, wurden die Duftprofile der gleichen Maiskultivaren über einen Zeitraum von drei Jahren auf zwei verschiedenen Feldern analysiert. Mit Ausnahme eines fehlenden Sesquiterpenes wurden die bereits für die Laborversuche beschriebenen Duftstoffe auch im Feld erfaßt. Dabei konnten wiederum qualitative und quantitative Unterschiede in der Abgabe volatiler Stoffe zwischen MON810 und der entsprechenden Kontrolllinie gefunden werden, wohingegen sich Pflanzen des Bt-events Bt176 lediglich quantitativ von ihren isogenen Kontrolllinien unterschieden. Im Verlauf der Feldversuche zeigte sich eine große Variabilität in der Abgabe volatiler Duftstoffe, insbesondere in Bezug auf den Verlauf innerhalb der Feldsaison und zwischen den Jahren. Im Gegensatz zum Jahr 2001, das keine qualitativen Unterschiede im Saisonverlauf aufwies, änderte sich das Duftstoffprofil der Maispflanzen im reproduktiven Stadium in den Jahren 2002 und 2003 dahingehend, daß anstelle der Sesquiterpenfraktion des Vorjahres zwei neue Sesquiterpene detektiert wurden. Diese Duftstoffe, als β- und α-Selinen identifiziert, wurden bisher noch nicht für kommerzielle Maissorten beschrieben. Die Komposition der Mono- und Homoterpene blieb hingegen gleich. Interessanterweise konnten diese drastische Änderung bei allen untersuchten Maiskultivaren sowie auf beiden Feldern gefunden werden. Eine vergleichbare Änderung im Duftstoffprofil von Pflanzen im Freiland wurde bisher noch nicht beschrieben.

Durch Fraßversuche mit zwölf aus den hier untersuchten und weiteren kommerziellen Maislinien bekannten Mono- und Sesquiterpenen sollten Effekte auf die Larvalentwicklung des Generalisten *Spodoptera littoralis* untersucht werden, um Rückschlüsse auf eine mögliche Funktion in der direkten Verteidigung der Pflanze schließen zu können. Während für die meisten Terpene positive oder neutrale Effekte auf

die Larvalentwicklung von *S. littoralis* gefunden werden konnten, beeinträchtigten vier der getesteten Terpene selbst in geringen Konzentrationen die Larvalentwicklung sehr deutlich. Das Sesquiterpen (*E*)-β-Farnesen verlängerte beispielsweise die Entwicklungszeit bis zur Verpuppung um zwei Tage, während Cycloisosativen und δ-Cadinen das Larvalgewicht signifikant verringerten. Ein viertes Sesquiterpen, β-Bisabolen, minderte den Schlupferfolg der adulten Schmetterlinge, nachdem die Larven diesem Stoff bis zur Verpuppung ausgesetzt waren.

Um die Ursache für die drastische Umstellung des Duftstoffprofils am Ende der Feldsaison 2002 und 2003 und eine mögliche Funktion der Sesquiterpene β- und α-Selinen zu ermitteln, wurden Maispflanzen den abiotischen Streßfaktoren Hitze, Trockenheit, Staunässe und erhöhte Ozonkonzentration sowie dem Herbizid Bromoxinyl ausgesetzt und die Duftstoffkomposition analysiert. Obwohl sich in allen Experimenten das Duftprofil qualitativ änderte, konnte die Emission beider Selinene nur durch die Applikation des Photosyntheseinhibitors Bromoxinyl auf Maispflanzen im Reproduktionsstadium induziert werden. Die organspezifische Verteilung dieser Sesquiterpene zeigte, daß beide Terpene lediglich von Kolbenhüll- und Tragblättern abgegeben wurden, nicht jedoch durch jüngere Blätter oder den männlichen Blütenstand.
Im Rahmen der vorliegenden Arbeit konnte erstmalig eine qualitative Änderung im Sesquiterpenprofil als Antwort auf die Behandlung mit einem Herbizid nachgewiesen werden. Die Emission von β-Selinen und α-Selinen nach Applikation des Photosyntheseinhibitors Bromoxinyl sowie deren organspezifische Abgabe weisen darauf hin, daß beide Sesquiterpene von der Pflanze als Antwort auf photooxidativen Stress emittiert werden und möglicherweise eine Schutzfunktion auf reproduktive Organe ausüben.

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Appendix

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II. Climatic conditions in the field near Halle

Data from the meteorological station next to the field site were obtained from the DWD (Deutscher Wetterdienst), Potsdam, Germany. Further informations are also available on http://www.koethen-wetter.de/. Temperature and relative humidity were additionally measured directly in the field after the end of the volatile collections.

Table A 1. Relative humidity (in %) and rainfall (in l/m³) in the months May until August in 2001

month	middle relative humidity			decade sum of rainfall (l/m²)		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	74.00	64.00	19.40	6.40	17.60	65.00
June	78.00	73.00	26.60	3.80	11.20	71.00
July	70.00	73.00	77.00	33.40	8.30	64.00
August	72.00	69.00	15.00	5.50	7.90	71.00

Table A 2. Temperatures (in °C) in the months May until August in 2001

month	middle day temperature			maximal day temperature			minimal day temperature		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	13.90	15.30	15.80	19.50	21.00	21.80	8.30	9.80	9.80
June	12.40	15.20	17.80	16.40	20.70	23.50	8.90	9.60	12.00
July	19.70	17.10	21.90	24.90	22.20	28.50	14.30	12.50	15.70
August	18.20	20.80	19.30	23.80	26.90	25.50	13.80	15.20	14.40

Table A 3. Temperatures (in °C) and mean relative humidity (in %) in the months June until August directly examined in the field 2001

month	mean temperature	maximal temperature	minimal temperature	relative humidity
June	26.40	39.60	14.60	42.25
July	30.33	34.30	26.90	38.50
August	32.98	27.90	42.80	20.05

Table A 4. Mean daily ozone concentrations (µg/m³) in the months May until August in 2001

Date	May	June	July	August
1	85.96	58.13	71.38	65.54
2	88.96	47.83	60.21	49.58
3	88.13	58.75	48.96	70.75
4	81.68	67.04	57.28	66.12
5	72.08	54.32	66.72	52.04
6	63.38	46.33	77.79	43.67
7	58.83	63.29	97.00	36.75
8	50.13	57.96	85.33	41.63
9	43.92	58.00	48.12	52.08
10	68.21	62.92	41.08	42.38
11	66.33	59.92	65.96	48.88
12	71.21	59.92	62.88	50.63
13	55.75	43.25	61.38	51.54
14	63.00	62.33	51.83	33.83
15	76.92	77.79	49.54	87.33
16	75.58	85.08	55.75	105.13
17	69.67	58.75	54.71	90.42
18	60.79	48.75	49.13	60.79
19	68.79	56.58	48.33	49.00
20	70.00	59.29	50.04	48.33
21	64.75	57.50	65.50	40.88
22	68.29	62.25	54.04	45.08
23	61.63	50.29	49.96	62.00
24	68.83	55.58	57.46	62.79
25	88.33	64.29	66.75	72.13
26	71.71	76.58	82.29	90.63
27	76.67	68.63	79.25	72.33
28	78.75	93.04	66.79	52.71
29	28.79	70.33	75.71	54.04
30	72.46	67.42	76.88	45.17
31	55.25		89.42	51.00

Table A 5. Maximum daily ozone concentrations (µg/m³) in the months May until August in 2001

Date	May	June	July	August
1	91.00	63.00	81.00	74.00
2	93.00	52.00	61.00	60.00
3	93.00	69.00	56.00	75.00
4	93.00	68.00	60.00	69.00
5	78.00	62.00	76.00	59.00
6	70.00	54.00	84.00	48.00
7	60.00	71.00	100.00	41.00
8	58.00	63.00	96.00	46.00
9	60.00	64.00	65.00	55.00
10	72.00	71.00	61.00	46.00
11	71.00	68.00	71.00	51.00
12	74.00	69.00	64.00	55.00
13	66.00	56.00	62.00	62.00
14	70.00	72.00	63.00	65.00
15	83.00	83.00	56.00	106.00
16	81.00	93.00	58.00	108.00
17	83.00	74.00	61.00	99.00
18	67.00	53.00	59.00	74.00
19	72.00	64.00	52.00	61.00
20	72.00	65.00	58.00	61.00
21	72.00	62.00	70.00	43.00
22	72.00	71.00	60.00	61.00
23	68.00	54.00	54.00	64.00
24	82.00	60.00	63.00	74.00
25	97.00	77.00	75.00	76.00
26	76.00	80.00	86.00	99.00
27	92.00	82.00	81.00	93.00
28	103.00	105.00	78.00	60.00
29	58.00	78.00	85.00	57.00
30	77.00	73.00	88.00	54.00
31	68.00		93.00	62.00

Table A 6. Relative humidity (in %) and rainfall (in l/m³) in the months May until August in 2002

month	middle relative humidity			decade sum of rainfall (l/m²)		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	81.00	75.00	79.00	26.80	7.30	32.30
June	78.00	80.00	70.00	11.70	12.00	2.00
July	74.00	82.00	71.00	28.40	84.20	7.80
August	83.00	78.00	76.00	74.30	27.70	0.00

Table A 7. Temperatures (in °C) in the months May until August in 2002

month	middle day temperature			maximal day temperature			minimal day temperature		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	13.60	15.90	15.10	18.20	21.20	20.70	9.70	11.00	10.50
June	16.20	18.60	17.20	21.00	23.90	22.50	12.10	14.10	12.40
July	18.20	17.70	19.70	23.80	22.10	24.20	12.90	14.00	14.00
August	19.10	20.40	21.00	24.80	25.50	27.10	15.20	15.90	15.60

Table A 8. Temperatures (in °C) and mean relative humidity (in %) in the months June until August, directly examined in the field 2002

month	mean temperature	maximal temperature	minimal temperature	relative humidity
June	23.7	28.00	20.00	47.64
July	25.48	30.9	18.10	42.55
August	21.73	26.5	16.00	51.75

Table A 9. Mean daily ozone concentrations (µg/m³) in the months May until August in 2002

Date	May	June	July	August
1	67.92	64.08	73.54	77.50
2	62.54	61.58	47.08	82.58
3	45.88	74.79	47.21	73.25
4	55.60	79.44	48.28	74.40
5	42.20	103.92	55.68	63.32
6	29.83	103.54	54.79	51.50
7	32.25	66.17	40.13	67.42
8	54.88	43.25	50.54	57.83
9	92.96	51.68	88.32	68.28
10	99.92	45.46	107.96	69.38
11	74.21	46.92	72.00	64.50
12	65.83	49.88	48.54	77.75
13	77.67	39.83	76.88	86.96
14	61.63	41.67	72.08	55.92
15	69.04	41.33	61.29	34.75
16	62.21	66.96	73.92	40.13
17	67.25	63.46	65.75	58.46
18	78.13	77.96	44.67	72.79
19	77.00	106.29	42.79	83.38
20	42.46	77.25	44.54	104.42
21	59.04	87.92	66.88	110.63
22	81.54	70.88	77.88	80.79
23	90.96	80.04	58.25	65.96
24	72.71	68.46	60.25	70.25
25	65.54	72.33	50.04	78.63
26	56.54	83.75	46.38	85.63
27	45.42	79.04	38.08	99.54
28	55.04	71.46	70.13	84.33
29	54.63	66.88	76.46	86.50
30	63.92	68.58	97.33	89.00
31	61.29		101.33	64.00

Table A 10. Maximum daily ozone concentrations (µg/m³) in the months May until August in 2002

Date	May	June	July	August
1	77.00	67.00	83.00	93.00
2	77.00	66.00	55.00	89.00
3	54.00	77.00	52.00	82.00
4	60.00	92.00	55.00	83.00
5	45.00	110.00	62.00	68.00
6	41.00	114.00	61.00	67.00
7	39.00	80.00	44.00	74.00
8	76.00	52.00	72.00	62.00
9	100.00	59.00	101.00	73.00
10	103.00	58.00	115.00	75.00
11	92.00	56.00	94.00	74.00
12	74.00	58.00	54.00	89.00
13	82.00	43.00	86.00	94.00
14	70.00	48.00	86.00	71.00
15	72.00	48.00	70.00	40.00
16	69.00	76.00	79.00	54.00
17	73.00	72.00	80.00	63.00
18	82.00	102.00	46.00	76.00
19	86.00	124.00	44.00	93.00
20	56.00	86.00	60.00	114.00
21	68.00	92.00	78.00	115.00
22	93.00	82.00	87.00	99.00
23	97.00	88.00	63.00	71.00
24	76.00	79.00	65.00	73.00
25	70.00	73.00	52.00	82.00
26	62.00	94.00	51.00	91.00
27	53.00	88.00	58.00	106.00
28	62.00	79.00	76.00	99.00
29	63.00	68.00	83.00	94.00
30	69.00	77.00	103.00	95.00
31	67.00		103.00	75.00

Table A 11. Relative humidity (in %) and rainfall (in l/m³) in the months May until August in 2003

month	middle relative humidity			decade sum of rainfall (l/m²)		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	66.00	76.00	70.00	3.00	45.50	8.50
June	66.00	68.00	63.00	60.00	7.90	2.10
July	71.00	57.00	68.00	8.00	0.10	8.60
August	55.00	56.00	66.00	0.00	10.00	14.20

Table A 12. Temperatures (in °C) in the months May until August in 2003

month	middle day temperature			maximal day temperature			minimal day temperature		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	15.20	12.50	17.00	21.30	18.30	22.90	8.70	7.90	11.20
June	21.30	18.40	19.10	28.10	23.80	25.10	14.10	13.60	12.20
July	17.30	22.00	21.30	21.90	28.10	28.20	13.70	13.70	15.80
August	24.00	21.20	17.50	31.90	28.10	23.30	16.40	15.30	12.70

Table A 13. Temperatures (in °C) and mean relative humidity (in %) in the months June until August, directly examined in the field 2003

month	mean temperature	maximal temperature	minimal temperature	relative humidity
June	26.70	30.00	24.70	31.18
July	30.18	32.90	25.40	21.38
August	27.90	34.00	23.30	22.08

Table A 14. Mean daily ozone concentrations (µg/m³) in the months May until August in 2003

Date	May	June	July	August
1	70.29	70.04	92.92	73.63
2	74.75	83.54	69.67	73.58
3	89.25	99.33	66.50	88.46
4	75.84	102.20	76.24	109.68
5	91.16	105.40	64.88	111.92
6	87.08	84.79	64.71	99.67
7	70.67	71.67	68.88	93.38
8	73.88	86.13	70.63	90.29
9	92.40	96.68	71.72	95.20
10	66.00	71.29	68.46	99.21
11	60.04	94.25	60.63	83.63
12	57.04	82.79	84.04	73.88
13	58.79	106.04	67.04	121.71
14	68.33	80.79	56.83	122.50
15	67.46	86.88	83.38	79.71
16	54.38	79.13	87.38	73.42
17	73.29	80.67	116.00	69.42
18	70.08	89.54	80.21	89.92
19	61.38	80.13	70.92	85.25
20	71.42	71.54	89.42	74.83
21	79.08	80.79	99.63	68.29
22	61.79	75.00	88.58	80.46
23	50.83	71.38	103.08	63.04
24	50.13	100.67	93.13	56.58
25	82.00	86.83	93.96	54.58
26	81.63	75.21	78.92	60.38
27	87.21	73.50	61.54	71.08
28	96.96	84.33	71.33	61.50
29	98.79	91.04	74.42	65.63
30	104.38	113.00	68.29	67.08
31	80.67		81.75	66.04

Table A 15. Maximum daily ozone concentrations (µg/m³) in the months May until August in 2003

Date	May	June	July	August
1	87.00	76.00	108.00	77.00
2	88.00	92.00	74.00	79.00
3	97.00	105.00	71.00	103.00
4	86.00	112.00	79.00	114.00
5	96.00	113.00	70.00	115.00
6	96.00	92.00	71.00	109.00
7	74.00	80.00	73.00	98.00
8	84.00	92.00	76.00	93.00
9	101.00	107.00	77.00	98.00
10	75.00	80.00	73.00	105.00
11	66.00	102.00	76.00	92.00
12	65.00	96.00	93.00	87.00
13	70.00	115.00	72.00	145.00
14	72.00	92.00	74.00	149.00
15	69.00	93.00	87.00	92.00
16	61.00	83.00	101.00	76.00
17	77.00	85.00	125.00	78.00
18	78.00	99.00	105.00	94.00
19	63.00	85.00	81.00	90.00
20	79.00	77.00	95.00	80.00
21	82.00	83.00	109.00	73.00
22	71.00	82.00	98.00	86.00
23	59.00	92.00	110.00	78.00
24	67.00	105.00	97.00	61.00
25	87.00	94.00	99.00	57.00
26	83.00	78.00	91.00	67.00
27	93.00	83.00	66.00	78.00
28	100.00	87.00	77.00	66.00
29	101.00	108.00	77.00	70.00
30	110.00	117.00	76.00	73.00
31	104.00		87.00	72.00

III. Climatic conditions in the field near Kitzingen

Data on climatic conditions of the region near the field site were available from Bayerisches Landesamt für Umwelt (website http://www.bayern.de/lfu/index.html) and the emission register (website http://interl.bayern.de/emissionskataster/php/start.php). Ozone concentrations were only available for Würzburg.

Table A 16. Relative humidity (in %) and rainfall (in l/m³) in the months May until August in 2002

month	middle relative humidity			decade sum of rainfall		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	no data available	no data available	no data available	2.54	2.53	1.92
June	72.17	67.29	58.20	2.80	5.40	28.00
July	64.95	76.52	67.25	92.50	2.70	15.60
August	76.56	69.99	76.00	15.30	9.50	33.70

Table A 17. Temperatures (in °C) in the months May until August in 2002

month	middle day temperature			maximal day temperature			minimal day temperature		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	12.37	15.41	14.12	17.48	21.91	20.44	7.62	9.20	7.81
June	16.55	20.61	18.60	22.30	27.51	25.91	10.66	13.08	11.76
July	18.49	17.74	19.23	26.20	21.94	25.95	11.11	13.77	12.53
August	18.00	19.70	19.60	23.59	25.53	27.08	13.31	13.48	14.12

Table A 18. Temperatures (in °C) and mean relative humidity (in %) in August 2002, directly examined in the field

month	mean temperature	maximal temperature	minimal temperature	relative humidity
August	22.73	23.80	21.70	59.30

Table A 19. Mean daily ozone concentrations (µg/m³) in the months May until August in 2002

Date	May	June	July	August
1	69.00	71.00	82.00	64.00
2	52.00	72.00	47.00	61.00
3	34.00	66.00	52.00	56.00
4	39.00	68.00	42.00	67.00
5	49.00	67.00	53.00	60.00
6	34.00	45.00	49.00	43.00
7	49.00	43.00	50.00	58.00
8	62.00	67.00	57.00	46.00
9	79.00	64.00	72.00	57.00
10	79.00	49.00	52.00	85.00
11	68.00	43.00	53.00	66.00
12	52.00	46.00	56.00	65.00
13	39.00	41.00	46.00	55.00
14	60.00	39.00	40.00	40.00
15	63.00	51.00	53.00	35.00
16	50.00	65.00	45.00	45.00
17	73.00	60.00	34.00	61.00
18	80.00	67.00	27.00	61.00
19	51.00	96.00	52.00	53.00
20	50.00	69.00	62.00	72.00
21	41.00	57.00	70.00	48.00
22	60.00	79.00	54.00	40.00
23	53.00	67.00	47.00	50.00
24	53.00	59.00	36.00	50.00
25	46.00	76.00	24.00	57.00
26	46.00	71.00	24.00	61.00
27	32.00	76.00	45.00	50.00
28	47.00	65.00	62.00	39.00
29	43.00	60.00	58.00	46.00
30	58.00	68.00	52.00	46.00
31	70.00		80.00	48.00

Table A 20. Maximum daily ozone concentrations (µg/m³) in the months May until August in 2002

Date	May	June	July	August
1	95.00	117.00	125.00	102.00
2	73.00	105.00	73.00	102.00
3	47.00	115.00	92.00	117.00
4	44.00	127.00	58.00	98.00
5	67.00	95.00	107.00	107.00
6	55.00	71.00	84.00	98.00
7	100.00	69.00	89.00	105.00
8	100.00	102.00	132.00	110.00
9	123.00	100.00	154.00	123.00
10	107.00	87.00	111.00	112.00
11	105.00	67.00	99.00	85.00
12	87.00	83.00	113.00	86.00
13	103.00	66.00	61.00	77.00
14	92.00	88.00	55.00	64.00
15	105.00	104.00	83.00	76.00
16	124.00	114.00	77.00	100.00
17	159.00	125.00	60.00	116.00
18	123.00	149.00	64.00	126.00
19	84.00	144.00	96.00	135.00
20	93.00	152.00	144.00	132.00
21	93.00	95.00	129.00	72.00
22	118.00	148.00	77.00	103.00
23	73.00	108.00	98.00	112.00
24	100.00	101.00	49.00	120.00
25	70.00	116.00	68.00	102.00
26	90.00	136.00	56.00	113.00
27	55.00	128.00	97.00	100.00
28	82.00	80.00	120.00	88.00
29	80.00	85.00	121.00	121.00
30	107.00	111.00	128.00	127.00
31	123.00		162.00	119.00

Table A 21. Relative humidity (in %) and rainfall (in l/m³) in the months May until August in 2003

month	middle relative humidity			decade sum of rainfall		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	57.79	70.61	68.61	23.70	42.10	10.60
June	59.71	62.3	49.14	5.00	23.10	7.70
July	64.80	46.62	6262.88	17.20	7.20	22.00
August	44.77	46.22	56.51	13.70	16.50	17.40

Table A 22. Temperatures (in °C) in the months May until August in 2003

month	middle day temperature			maximal day temperature			minimal day temperature		
	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade	1. decade	2. decade	3. decade
May	16.37	12.51	16.96	23.97	17.86	22.65	7.82	7.32	11.55
June	21.92	20.82	20.52	30.43	27.79	28.58	13.62	14.11	11.55
July	17.56	21.72	20.77	23.02	29.54	27.62	15.52	13.32	14.31
August	26.25	23.23	18.28	35.00	30.99	25.55	16.01	15.29	10.68

Table A 23. Temperatures (in °C) and mean relative humidity (in %) in the months July and August, directly examined in the field 2003

month	mean temperature	maximal temperature	minimal temperature	relative humidity
July	30.20	32.90	26.60	20.37
August	32.40	35.30	29.90	14.67

Table A 24. Mean daily ozone concentrations (µg/m³) in the months May until August in 2003

Date	May	June	July	August
1	85.00	71.00	62.00	84.00
2	60.00	78.00	66.00	89.00
3	82.00	66.00	65.00	110.00
4	67.00	72.00	50.00	118.00
5	69.00	73.00	60.00	123.00
6	61.00	83.00	71.00	114.00
7	70.00	84.00	76.00	106.00
8	71.00	85.00	96.00	86.00
9	74.00	86.00	90.00	107.00
10	54.00	76.00	84.00	139.00
11	61.00	84.00	76.00	90.00
12	63.00	85.00	85.00	91.00
13	59.00	89.00	78.00	118.00
14	70.00	81.00	72.00	120.00
15	48.00	98.00	88.00	87.00
16	54.00	91.00	96.00	85.00
17	51.00	102.00	66.00	102.00
18	60.00	79.00	53.00	82.00
19	44.00	58.00	67.00	71.00
20	67.00	77.00	91.00	80.00
21	61.00	84.00	92.00	91.00
22	44.00	73.00	90.00	107.00
23	43.00	85.00	96.00	61.00
24	58.00	102.00	77.00	71.00
25	76.00	90.00	56.00	62.00
26	54.00	86.00	47.00	75.00
27	69.00	102.00	58.00	55.00
28	67.00	108.00	58.00	54.00
29	88.00	94.00	75.00	73.00
30	84.00	85.00	71.00	43.00
31	49.00		74.00	58.00

Table A 25. Maximum daily ozone concentrations (µg/m³) in the months May until August in 2003

Date	May	June	July	August
1	106.00	141.00	83.00	139.00
2	102.00	141.00	89.00	168.00
3	105.00	141.00	95.00	177.00
4	126.00	158.00	73.00	202.00
5	128.00	165.00	87.00	199.00
6	114.00	118.00	96.00	173.00
7	105.00	147.00	120.00	182.00
8	125.00	130.00	166.00	217.00
9	104.00	118.00	144.00	209.00
10	97.00	147.00	116.00	222.00
11	115.00	136.00	135.00	145.00
12	111.00	175.00	110.00	194.00
13	91.00	154.00	102.00	222.00
14	91.00	151.00	107.00	190.00
15	87.00	138.00	139.00	112.00
16	122.00	136.00	174.00	134.00
17	93.00	169.00	87.00	159.00
18	84.00	119.00	97.00	109.00
19	83.00	72.00	126.00	110.00
20	99.00	123.00	147.00	132.00
21	76.00	112.00	129.00	154.00
22	79.00	125.00	144.00	152.00
23	106.00	155.00	173.00	106.00
24	116.00	134.00	145.00	110.00
25	89.00	119.00	121.00	106.00
26	78.00	128.00	88.00	135.00
27	95.00	148.00	106.00	84.00
28	102.00	144.00	97.00	117.00
29	119.00	155.00	126.00	118.00
30	138.00	162.00	147.00	72.00
31	87.00		141.00	83.00

IV. Laboratory Data

Table A 26. Volatile emission of 4-week-old plants after feeding by *Spodoptera littoralis* on the corn cultivars Nobilis (isogenic) and Novelis (transgenic)
Mean and standard deviations are shown for the control and feeding by *Spodoptera littoralis*; N = 6.

volatile compound (ng/h/g fresh weight)	**control**		***Spodoptera littoralis***	
	Nobilis	**Novelis**	**Nobilis**	**Novelis**
(*Z*)-3-hexen-1-yl acetate	0.518 ± 0.301	14.255 ± 4.607	42.691 ± 7.786	37.529 ± 10.705
limonene	0.133 ± 0.093	1.725 ± 0.576	8.599 ± 1.747	1.385 ± 0.763
(*E*)-β-ocimene	0.060 ± 0.038	2.115 ± 0.612	12.798 ± 2.012	3.262 ± 0.718
linalool	1.087 ± 0.268	17.222 ± 4.191	35.158 ± 8.268	118.267 ± 22.628
DMNT	11.640 ± 1.310	7.853 ± 2.518	114.039 ± 20.265	70.933 ± 24.924
α-ylangene	0.003 ± 0.001	0.000 ± 0.000	0.089 ± 0.032	0.000 ± 0.000
(*E*)-β-caryophyllene	7.664 ± 1.299	3.759 ± 1.437	144.239 ± 20.542	57.157 ± 11.100
(*E*)-α-bergamotene	5.625 ± 2.129	2.269 ± 0.940	35.550 ± 10.633	22.916 ± 8.814
(*E*)-β-farnesene	23.361 ± 6.011	3.639 ± 1.649	135.417 ± 21.848	34.727 ± 8.793
(*E,E*)-α-farnesene	9.212 ± 4.540	5.864 ± 1.580	95.359 ± 19.576	35.633 ± 4.641
γ-cadinene	0.000 ± 0.000	0.059 ± 0.023	0.351 ± 0.117	0.0868 ± 0.0242
δ-cadinene	0.185 ± 0.096	0.254 ± 0.134	0.348 ± 0.085	0.337 ± 0.0856
β-sesquiphellandrene	4.010 ± 2.571	0.000 ± 0.000	8.424 ± 2.693	0.000 ± 0.000
total volatiles	63.498 ± 11.753	59.014 ± 10.624	633.063 ± 77.910	382.234 ± 72.027

Table A 27. Volatile emission of 4-week-old plants after feeding by different insect species on the corn cultivars Prelude (isogenic) and Valmont (transgenic)
Mean and standard deviations are shown for the control, feeding by *Spodoptera littoralis*, *Ostrinia nubilalis* and *Agrotis segetum*; Prel=Prelude; Val=Valmont; N = 6.

volatile compound (ng/h/g fresh weight)	control		*Spodoptera littoralis*		*Agrotis segetum*		*Ostrinia nubilalis*	
	Prel	**Val**	**Prel**	**Val**	**Prel**	**Val**	**Prel**	**Val**
(*Z*)-3-hexen-1-yl acetate	0.557± 0.099	0.336 ± 0.0829	26.240 ± 4.215	21.339 ± 0.238	1.930 ± 0.724	3.272 ± 1.237	1.201 ± 0.106	0.985 ± 0.303
limonene	0.061 ± 0.009	0.059 ± 0.007	0.308 ± 0.025	0.356 ± 0.044	0.096 ± 0.044	0.903 ± 0.845	0.012 ± 0.002	0.037 ± 0.016
(*E*)-β-ocimene	0.603 ± 0.203	0.195 ± 0.058	4.377 ± 0.975	8.242 ± 0.899	0.563 ± 0.087	0.620 ± 0.283	0.182 ± 0.035	0.206 ± 0.059
linalool	0.935 ± 0.212	1.190 ± 0.171	103.107± 28.153	149.757± 22.134	8.764 ± 2.789	20.904 ± 10.526	3.378 ± 0.845	2.289± 0.321
DMNT	0.547 ± 0.156	0.951 ± 0.298	39.490 ± 6.899	68.769 ± 7.200	1.936 ± 0.444	2.961 ± 1.166	2.560 ± 0.490	1.461 ± 0.270
α-ylangene	0.003 ± 0.0002	0.006 ± 0.001	0.020 ± 0.005	0.031 ± 0.004	0.011 ± 0.001	0.013 ± 0.001	0.001 ± 0.0001	0.001 ± 0.0001
cycloiso-sativene	1.171 ± 0.198	2.694 ± 0.358	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.260 ± 0.024	0.249 ± 0.079
(*E*)-β-caryo-phyllene	0.452 ± 0.140	0.967 ± 0.187	4.206 ± 1.007	1.443 ± 0.224	0.540 ± 0.059	0.573 ± 0.055	0.331 ± 0.083	1.965 ± 0.306
(*E*)-α-berga-motene	0.000 ± 0.000	0.000 ± 0.000	78.113 ± 16.642	96.493± 8.406	1.197 ± 0.467	3.232 ± 2.299	3.236 ± 0.617	1.572 ± 0.312
(*E*)-β-farnesene	0.330 ± 0.137	0.511 ± 0.119	215.827± 44.948	276.183± 27.997	2.780 ± 0.827	7.077 ± 4.633	9.586 ± 1.740	5.008 ± 0.808
(*E,E*)-α-farnesene	4.695 ± 1.743	5.151 ± 1.021	44.211 ± 11.749	69.763± 8.581	11.316 ± 2.367	13.080 ± 3.625	2.435 ± 0.304	3.975 ± 0.757
β-bisabolene	0.000 ± 0.000	0.000 ± 0.000	5.065 ± 1.109	6.007 ± 0.549	0.000 ± 0.000	0.000± 0.000	0.000 ± 0.000	0.000 ± 0.000
γ-cadinene	0.011 ± 0.003	0.030 ± 0.005	0.059 ± 0.005	0.081 ± 0.012	0.031 ± 0.003	0.029 ± 0.004	0.003 ± 0.001	0.004 ± 0.0009
δ-cadinene	0.0410± 0.0121	1.261 ± 0.162	0.052 ± 0.007	0.117± 0.019	0.0221 ± 0.005	0.019 ± 0.005	0.000 ± 0.000	0.000 ± 0.000
β-sesqui-phellandrene	0.000 ± 0.000	0.000 ± 0.000	17.994 ± 3.903	20.427± 1.934	0.234 ± 0.107	0.608± 0.313	0.743 ± 0.144	0.397 ± 0.075
total volatiles	9.407 ± 2.255	13.351 ± 1.661	539.444± 101.599	719.533± 51.608	29.418 ± 6.518	53.292 ± 21.099	23.929 ± 3.969	18.152 ± 2.997

Table A 28. Volatile emission of 4-week-old plants after feeding by different insect species on the corn cultivars Antares (isogenic) and Navares (transgenic)
Mean and standard deviations are shown for the control and feeding by *Spodoptera littoralis* and *Ostrinia nubilalis*; N = 6.

volatile compound (ng/h/g fresh weight)	Control		*Spodoptera littoralis*		*Ostrinia nubilalis*	
	Antares	**Navares**	**Antares**	**Navares**	**Antares**	**Navares**
β-myrcene	0.000 ± 0.000	0.000 ± 0.000	0.428 ± 0.0573	0.598 ± 0.077	0.000 ± 0.000	0.000 ± 0.000
limonene	0.000 ± 0.000	0.000 ± 0.000	1.812 ± 0.298	3.228 ± 0.366	0.965 ± 0.250	1.192 ± 0.255
(*E*)-β-ocimene	0.000 ± 0.000	0.000 ± 0.000	3.098 ± 0.640	4.649 ± 0.902	0.000 ± 0.000	0.000 ± 0.000
linalool	1.122 ± 0.326	3.361 ± 0.561	94.379 ± 13.439	108.664 ± 21.288	7.158 ± 2.514	5.416 ± 2.022
DMNT	0.602 ± 0.140	0.858 ± 0.199	45.976 ± 10.489	42.245 ± 9.799	1.130 ± 0.380	1.267 ± 0.384
α-ylangene	0.001 ± 0.0001	0.001 ± 0.0002	0.020 ± 0.002	0.068 ± 0.014	0.000 ± 0.000	0.000 ± 0.000
cycloisosativene	0.000 ± 0.000	0.000 ± 0.000	8.913 ± 0.969	31.176 ± 7.007	0.000 ± 0.000	0.000 ± 0.000
(*E*)-β-caryophyllene	0.429 ± 0.114	0.562 ± 0.165	18.433 ± 3.099	47.958 ± 8.478	2.720 ± 1.216	0.662 ± 0.222
(*E*)-α-bergamotene	0.414 ± 0.066	0.662 ± 0.173	1.990 ± 0.403	4.292 ± 0.570	2.811 ± 1.439	1.300 ± 0.151
(*E*)-β-farnesene	1.038 ± 0.476	2.047 ± 0.715	9.018 ± 1.561	18.675 ± 2.426	1.917 ± 0.485	2.310 ± 0.488
(*E*,*E*)-α-farnesene	0.000 ± 0.000	0.000 ± 0.000	22.779 ± 4.612	28.597 ± 8.105	0.313 ± 0.218	0.584 ± 0.383
γ-cadinene	0.000 ± 0.000	0.000 ± 0.000	0.057 ± 0.009	0.104 ± 0.017	0.013 ± 0.003	0.007 ± 0.001
δ-cadinene	0.000 ± 0.000	0.000 ± 0.000	0.149 ± 0.029	0.566 ± 0.126	0.014 ± 0.002	0.009 ± 0.001
total volatiles	3.606 ± 0.809	7.491 ± 0.721	207.052 ± 32.286	290.819 ± 38.468	19.489 ± 7.966	12.747 ± 2.054

V. Field Data

Table A 29. Volatile emission of the corn cultivars Nobilis (isogenic) and Novelis (transgenic) in the field near Halle in 2001
Mean and standard deviations are shown; N = 6.

volatile compound (ng/h/plant)	Nobilis			Novelis		
	June	July	August	June	July	August
limonene	100.159 ± 22.377	108.334 ± 17.373	92.566 ± 11.176	206.339 ± 58.713	358.018 ± 74.678	203.111 ± 43.482
(*E*)-β-ocimene	87.976 ± 23.817	31.126 ± 10.452	87.187 ± 20.010	113.943 ± 33.102	108.440 ± 32.441	61.706 ± 15.058
linalool	700.799 ± 158.957	678.183 ± 150.136	869.639 ± 203.323	1107.130 ± 233.631	1193.776 ± 247.754	453.204 ± 119.937
DMNT	333.125 ± 74.988	337.853 ± 87.164	526.076 ± 160.977	523.345 ± 99.117	416.349 ± 90.758	192.047 ± 52.989
α-ylangene	3.465 ± 0.919	7.021 ± 1.474	6.015 ± 1.880	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
(*E*)-β-caryophyllene	792.502 ± 198.077	963.069 ± 220.374	612.357 ± 165.131	1126.149 ± 222.331	620.545 ± 146.934	296.897 ± 105.563
(*E*)-α-bergamotene	315.028 ± 94.269	488.283 ± 134.224	358.024 ± 109.503	307.970 ± 58.997	162.652 ± 54.635	84.234 ± 13.240
(*E*)-β-farnesene	342.964 ± 73.548	230.598 ± 18.660	516.209 ± 114.508	482.161 ± 88.282	320.063 ± 96.865	189.315 ± 34.978
(*E*,*E*)-α-farnesene	360.672 ± 80.583	341.647 ± 58.032	183.483 ± 45.160	351.905 ± 82.058	221.711 ± 99.574	113.978 ± 22.048
γ-cadinene	16.959 ± 5.109	18.754 ± 6.589	22.635 ± 5.699	25.945 ± 4.718	14.381 ± 5.097	4.834 ± 1.561
δ-cadinene	28.311 ± 6.814	35.609 ± 10.767	40.305 ± 14.384	31.012 ± 6.666	16.486 ± 5.516	16.615 ± 4.128
total amount	3081.961 ± 709.492	3240.477 ± 560.976	3314.496 ± 735.718	4275.898 ± 769.722	3432.421 ± 705.146	1615.941 ± 347.476

Table A 30. Volatile emission of the corn cultivars Nobilis (isogenic) and Novelis (transgenic) in the fields near Halle and Kitzingen in august 2002 and 2003
Mean and standard deviations are shown; N = 6.

volatile compound (ng/h/plant)	Nobilis			Novelis		
	Halle 2002	Kitzingen 2002	Halle 2003	Halle 2002	Kitzingen 2002	Halle 2003
β-myrcene	0.000 ± 0.000	5.734 ± 3.061	0.000 ± 0.000	0.000 ± 0.000	2.448 ± 0.862	0.000 ± 0.000
limonene	248.055 ± 62.571	164.702 ± 61.490	126.296 ± 48.595	88.065 ± 22.871	78.149 ± 18.899	175.203 ± 34.152
(*E*)-β-ocimene	134.220 ± 48.611	0.000 ± 0.000	167.742 ± 56.881	131.064 ± 39.484	0.000 ± 0.000	296.896 ± 84.184
linalool	346.397 ± 251.330	421.560 ± 116.916	510.372 ± 108.469	61.908 ± 42.285	407.726 ± 109.470	538.966 ± 60.628
DMNT	371.111 ± 59.842	828.940 ± 94.030	805.171 ± 121.657	252.015 ± 58.741	428.114 ± 114.402	1272.799 ± 229.440
α-ylangene	0.000 ± 0.000	0.659 ± 0.295	0.512 ± 0.197	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
(*E*)-α-bergamotene	0.000 ± 0.000	0.000 ± 0.000	300.125 ± 110.157	0.000 ± 0.000	0.000 ± 0.000	248.785 ± 83.160
(*E*)-β-farnesene	0.000 ± 0.000	0.000 ± 0.000	1034.426 ± 632.844	0.000 ± 0.000	0.000 ± 0.000	355.692 ± 95.217
β-selinene	668.531 ± 73.541	32.771 ± 5.845	38.184 ± 11.251	192.167 ± 69.895	37.758 ± 11.677	93.512 ± 19.188
α-selinene	197.139 ± 51.900	56.417 ± 5.597	52.732 ± 17.063	71.402 ± 24.071	48.407 ± 13.222	113.753 ± 18.940
β-bisabolene	0.000 ± 0.000	0.000 ± 0.000	110.251 ± 26.932	0.000 ± 0.000	0.000 ± 0.000	151.142 ± 20.855
total amount	1965.453 ± 413.749	1510.783 ± 162.761	3145.811 ± 865.422	796.621 ± 223.395	1002.604 ± 168.817	3246.749 ± 349.156

Table A 31. Volatile emission of the corn cultivars Prelude (isogenic) and Valmont (transgenic) in the field near Halle in 2002
Mean and standard deviations are shown; N = 6.

volatile compound (ng/h/plant)	Prelude			Valmont		
	June	July	August	June	July	August
β-myrcene	0.000 ± 0.000	109.742 ± 37.383	0.000 ± 0.000	0.000 ± 0.000	11.829 ± 4.639	0.000 ± 0.000
(*Z*)-3-hexen-1-yl acetate	118.239 ± 24.846	0.000 ± 0.000	0.000 ± 0.000	154.495 ± 41.572	0.000 ± 0.000	0.000 ± 0.000
limonene	30.399 ± 6.914	312.071 ± 84.604	81.731± 23.481	31.154 ± 9.563	267.146 ± 87.948	0.000 ± 0.000
(*E*)-β-ocimene	0.000 ± 0.000	397.355 ± 123.305	0.000 ± 0.000	0.000 ± 0.000	450.764 ± 104.532	0.000 ± 0.000
linalool	82.093 ± 26.422	559.839 ± 119.256	0.000 ± 0.000	82.239 ± 17.01	410.516 ± 119.293	0.000 ± 0.000
DMNT	127.979 ± 31.561	351.224 ± 102.635	92.404 ± 13.263	143.456 ± 53.443	271.747 ± 46.004	60.256 ± 14.488
α-ylangene	1.708 ± 0.423	1.181 ± 0.18	0.160± 0.060	1.341 ± 0.41	0.391 ± 0.134	0.322 ± 0.113
cycloisosativene	205.201± 55.789	0.000 ± 0.000	0.000 ± 0.000	165.647 ± 43.954	0.000 ± 0.000	0.000 ± 0.000
(*E*)-β-caryophyllene	319.890 ± 123.825	140.089 ± 48.559	0.000 ± 0.000	254.929 ± 77.848	183.383 ± 50.021	0.000 ± 0.000
(*E*)-α-bergamotene	0.000 ± 0.000	247.786 ± 75.489	0.000 ± 0.000	0.000 ± 0.000	186.551 ± 39.984	0.000 ± 0.000
(*E*)-β-farnesene	140.989 ± 65.805	143.835 ± 41.223	0.000 ± 0.000	84.273± 13.739	173.317 ± 63.400	0.000 ± 0.000
β-selinene	0.000 ± 0.000	0.000 ± 0.000	69.167 ± 9.694	0.000 ± 0.000	0.000 ± 0.000	106.45 ± 25.313
α-selinene	0.000 ± 0.000	0.000 ± 0.000	52.781 ± 7.981	0.000 ± 0.000	0.000 ± 0.000	90.449 ± 22.326
(*E*,*E*)-α-farnesene	185.810 ± 80.372	0.000 ± 0.000	0.000 ± 0.000	225.182 ± 59.516	0.000 ± 0.000	0.000 ± 0.000
γ-cadinene	8.815 ± 3.140	11.446 ± 2.126	0.000 ± 0.000	6.043 ± 1.114	7.943 ± 1.806	0.000 ± 0.000
δ-cadinene	18.544 ± 6.293	10.532 ± 2.074	0.000 ± 0.000	12.565± 3.276	23.343 ± 5.991	0.000 ± 0.000
total amount	1239.666 ± 310.687	2285.100 ± 246.903	296.242± 41.984	1161.324 ± 273.249	1986.929 ± 258.487	357.553 ± 92.369

Table A 32. Volatile emission of the corn cultivars Prelude (isogenic) and Valmont (transgenic) in the field near Halle in 2003
Mean and standard deviations are shown; N = 6.

volatile compound (ng/h/plant)	Prelude			Valmont		
	June	July	August	June	July	August
β-myrcene	3.067 ± 2.136	7.039 ± 1.386	4.483 ± 1.608	2.061 ± 1.582	8.374 ± 1.418	5.306 ± 3.339
(*Z*)-3-hexen-1-yl acetate	1681.161 ± 317.767	0.000 ± 0.000	0.000 ± 0.000	1947.371 ± 400.633	0.000 ± 0.000	0.000 ± 0.000
limonene	281.428 ± 32.874	225.336 ± 62.543	138.367 ± 78.984	240.941 ± 64.438	152.170 ± 52.350	101.873 ± 42.532
(*E*)-β-ocimene	0.000 ± 0.000	214.267 ± 61.409	109.641 ± 36.598	0.000 ± 0.000	312.132 ± 70.957	88.272 ± 41.429
linalool	604.170 ± 121.249	445.206 ± 120.698	263.687 ± 92.179	787.072 ± 235.422	540.112 ± 138.621	279.699 ± 60.686
DMNT	567.797 ± 165.194	547.566 ± 147.370	274.097 ± 120.055	973.977 ± 221.937	679.194 ± 70.319	294.034 ± 55.142
α-ylangene	14.804 ± 1.993	6.838 ± 2.329	2.505 ± 1.113	14.505 ± 2.436	11.050 ± 3.007	2.344 ± 0.776
(*E*)-β-caryophyllene	0.000 ± 0.000	1182.558 ± 458.425	0.000 ± 0.000	0.000 ± 0.000	326.330 ± 70.706	0.000 ± 0.000
(*E*)-α-bergamotene	0.000 ± 0.000	203.184 ± 59.374	0.000 ± 0.000	0.000 ± 0.000	462.800 ± 224.399	0.000 ± 0.000
(*E*)-β-farnesene	487.709 ± 131.208	422.084 ± 64.381	0.000 ± 0.000	357.689 ± 161.919	263.332 ± 103.272	0.000 ± 0.000
β-selinene	0.000 ± 0.000	59.987 ± 9.448	32.688 ± 16.496	0.000 ± 0.000	70.439 ± 13.423	93.915 ± 39.563
α-selinene	0.000 ± 0.000	76.284 ± 5.649	47.523 ± 17.665	0.000 ± 0.000	75.847 ± 29.737	90.754 40.195
(*E*,*E*)-α-farnesene	55.175 ± 29.518	0.000 ± 0.000	0.000 ± 0.000	139.607 ± 123.942	0.000 ± 0.000	0.000 ± 0.000
γ-cadinene	35.381 ± 10.812	15.480 ± 2.008	0.000 ± 0.000	24.924 ± 5.666	18.705 ± 7.524	0.000 ± 0.000
δ-cadinene	66.959 ± 28.504	24.569 ± 5.169	0.000 ± 0.000	36.676 ± 10.683	22.626 ± 6.941	0.000 ± 0.000
total amount	3852.684 ± 407.375	3430.395 ± 510.786	872.992 ± 285.578	4524.823 ± 766.689	2943.110 ± 556.478	956.195 ± 219.304

Table A 33. Volatile emission of the corn cultivars Antares (isogenic) and Navares (transgenic) in the field near Kitzingen in 2002 and 2003
Mean and standard deviations are shown; N = 6.

volatile compound (ng/h/plant)	Antares			Navares		
	08/ 2002	**07/ 2003**	**08/ 2003**	**08/ 2002**	**07/ 2003**	**08/ 2003**
β-myrcene	10.764 ± 1.733	10.125 ± 2.210	3.136 ± 0.526	11.376 ± 1.825	13.943 ± 3.280	1.448 ± 0.080
(*Z*)-3-hexen-1-yl acetate	0.000 ± 0.000	0.000 ± 0.000	253.065 ± 85.432	0.000 ± 0.000	0.000 ± 0.000	517.935 ± 132.475
limonene	360.382 ± 66.423	577.610 ± 116.700	167.022 ± 22.505	509.774 ± 95.480	686.116 ± 139.985	156.943 ± 28.270
(*E*)-β-ocimene	76.807 ± 20.244	0.000 ± 0.000	240.516 ± 16.845	55.483 ± 10.882	0.000 ± 0.000	308.552 ± 32.617
linalool	584.831± 70.669	611.842 ± 61.881	524.906 ± 50.122	652.693 ± 84.683	706.337 ± 115.512	598.721 ± 157.095
DMNT	258.461± 37.267	247.794 ± 26.974	866.564 ± 126.203	274.774 ± 33.744	416.111 ± 77.038	1162.638 ± 158.668
α-ylangene	0.709 ± 0.182	9.117 ± 1.930	0.204 ± 0.067	1.166 ± 0.273	9.118 ± 1.157	0.088 ± 0.040
(*E*)-β-caryophyllene	0.000 ± 0.000	244.675 ± 90.373	0.000 ± 0.000	0.000 ± 0.000	351.488 ± 102.179	0.000 ± 0.000
(*E*)-β-farnesene	19.206 ± 13.657	0.000 ± 0.000	0.000 ± 0.000	5.110 ± 4.075	0.000 ± 0.000	0.000 ± 0.000
β-selinene	43.093 ± 6.081	0.000 ± 0.000	21.132 ± 3.019	51.165 ± 5.953	0.000 ± 0.000	41.305 ± 11.619
α-selinene	84.407 ± 20.300	0.000 ± 0.000	52.951 ± 12.893	104.358 ± 20.141	0.000 ± 0.000	95.570 ± 23.673
γ-cadinene	0.000 ± 0.000	30.417 ± 9.531	0.000 ± 0.000	0.000 ± 0.000	17.156 ± 2.248	0.000 ± 0.000
δ-cadinene	0.000 ± 0.000	49.851 ± 4.263	0.000 ± 0.000	0.000 ± 0.000	29.398 ± 5.935	0.000 ± 0.000
total amount	1438.661 ± 158.227	1781.432 ± 249.838	2129.497 ± 252.658	1665.899 ± 231.977	2229.667 ± 400.389	2883.199 ± 399.608

Lebenslauf

Persönliche Daten:		
	Geburtstag:	27.03.1975
	Geburtsort:	Leipzig, Deutschland
	Familienstand:	ledig
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Dissertation:	seit Mai 2001	MPI für Chemische Ökologie, Abteilung Biochemie -Thema: „The effect of Bt-transformation and various environmental factors on the volatile emission of maize: Potential influence on direct and indirect defense against herbivores”

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Hiermit erkläre ich, daß:

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- ich diese Arbeit, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

Jena, Mai 2006 Christine Zipfel

WISSENSCHAFTLICHE PUBLIKATIONEN UND VORTRÄGE

Poster:

Statusseminar des BMBF zu Verbundprojekten von gentechnisch verändertem Raps, Kartoffeln und Mais, Juni 2004, Berlin: Riecht Bt-Mais anders?

SIP12-Symposium Insect-Plant-Relationships August 2004, Berlin: The role of maize terpenes in direct defense against insect herbivores.

Vorträge:

Verbundtreffen „Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais“ *November 2002, Darmstadt:* Effekte von Bt-endotoxin auf die tritrophische Interaktion zwischen Mais, Nichtziel-Lepidopteren und deren Parasitoiden.

Verbundtreffen „Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais“ *November 2003, Göttingen:* Effekte von Bt-endotoxin auf die tritrophische Interaktion zwischen Mais, Nichtziel-Lepidopteren und deren Parasitoiden - neue Erkenntnisse.

Workshop mit Doktoranden vom MPI fürMolekulare Pflanzenphysiologie, Potsdam/ Golm, 25.04.02: Does abiotic/ biotic stress change the volatile profile of corn?

Thesen zur Dissertation

„The effect of Bt-transformation and various environmental factors on the volatile emission of maize: Potential influence on direct and indirect defense against herbivores"

Seit einigen Jahren ist bekannt, daß von Herbivoren befallene Pflanzen Duftstoffe emittieren, die von Prädatoren oder Parasitoiden wahrgenommen und genutzt werden, um ihre Beute bzw. ihren Wirtsorganismus auf den befallenen Pflanzen zu finden. Diese Duftstoffabgabe dient als indirekte Verteidigungsstrategie der Pflanze, die auch für Mais beschrieben wurde. Darüber hinaus ist bekannt, daß Duftstoffe, insbesondere Terpene, als Fraßhemmstoffe oder Toxine fungieren und die Pflanze somit auch auf direktem Wege verteidigen können. Abiotische Faktoren wie z.B. Ozon, Hitze, Trockenheit oder Staunässe können das Muster volatiler Stoffe verändern und damit möglicherweise die Verteidigungsmechanismen der Pflanze beeinflussen. Die zunehmende Kulitvierung gentechnisch manipulierter Pflanzen wirft die Frage auf, ob der Einbau eines artfremden Gens neben den erwünschten Effekten auch unerwünschte Nebeneffekte haben könnte, wie beispielsweise die Änderung des Duftstoffprofils. Zur Beantwortung dieser Frage wurden im Rahmen der vorliegenden Arbeit die Emissionen der Duftstoffe analysiert, die von drei transgenen Maissorten sowie deren isogenen Kontrolllinien sowohl nach Befall mit unterschiedlichen Herbivoren als auch im unbehandelten Zustand unter Gewächshausbedingungen abgegeben wurden. Desweiteren wurden die gleichen Maissorten über einen Zeitraum von drei Jahren im Feld kultiviert und deren Duftstoffprofil untersucht. In Laborversuchen wurden Maisterpene hinsichtlich deren Wirkung auf Larven des Herbivoren *Spodoptera littoralis* getestet. Desweiteren wurden Maispflanzen mit vier abiotischen Streßfaktoren sowie einem Herbizid behandelt und das Muster volatiler Duftstoffe analysiert.

1. Der Einbau des Bt-kodierenden Gens in Mais verändert das Duftstoffprofil der Pflanzen im Vergleich zur isogenen Kontrolllinie quantitativ und z. T. auch qualitativ in Abhängigkeit der Kultivare und des Bt-events.

2. Transgene und deren isogene Linien verhalten sich unterschiedlich hinsichtlich des Duftprofils nach Befall mit den Herbivoren *Ostrinia nubilalis*, *Agrotis segetum* oder *Spodoptera littoralis*. Unabhängig vom Bt-Einbau induzieren die Larven dieser verschiedenen Arten qualitativ und quantitativ unterschiedliche Duftstoffprofile.

3. Im Freiland schwankt die Duftstoffabgabe durch Maispflanzen sowohl zwischen einzelnen Jahren als auch im Verlauf einer Wachstumsperiode stark.

4. Als Bestandteile der Nahrung können Cycloisosativen, δ-Cadinen, (*E*)-β-Farnesen sowie β-Bisabolen, Terpene des Duftstoffprofils der hier untersuchten Maissorten, die Larvalentwicklung des Herbivoren *Spodoptera littoralis* negativ beeinflussen, was auf eine Rolle dieser Stoffe in der direkten Verteidigung der Maispflanzen hinweist.

5. Photooxidativer Streß, hervorgerufen durch Applikation des Photosyntheseinhibitors Bromoxinyl, verändert das Duftstoffprofil von Maispflanzen im reproduktiven Stadium dahingehend, daß zwei Sesquiterpene, α-Selinen und β-Selinen, als Haupbestandteile des Duftbouquet neu gebildet werden, und diese die anderen Sesquiterpene weitestgehend ersetzen. Die Emission beider Selinene erfolgt nur durch Tragblatt und Kolbenhüllblätter und unterliegt damit einer organspezifischen Verteilung.